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# MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

VOLUME XXVII, 1935

WITH 44 PLATES AND 359 TEXT FIGURES

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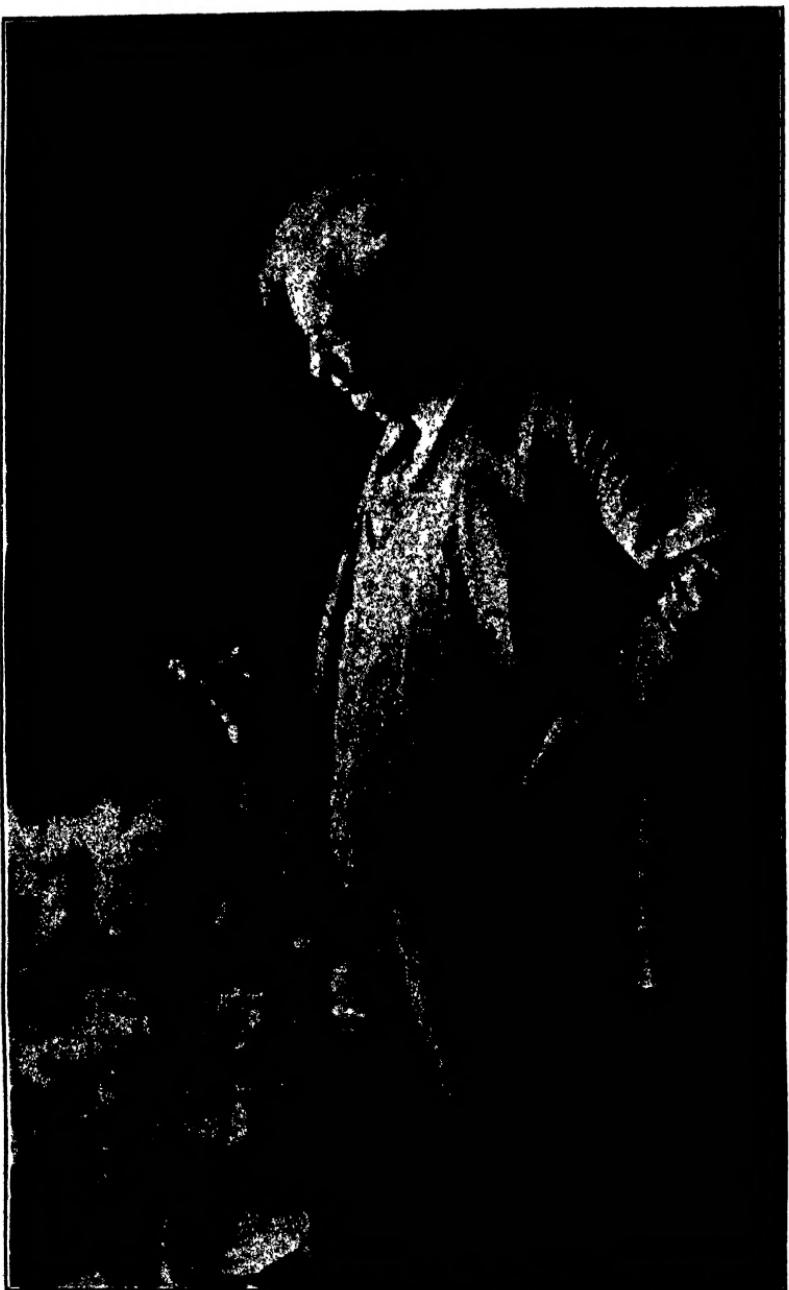
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FRANK LINCOLN STEVENS

# MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

VOL. XXVII JANUARY-FEBRUARY, 1935 No. 1

## FRANK LINCOLN STEVENS

L. R. TEHON

(WITH PORTRAIT)

Frank Lincoln Stevens,<sup>1</sup> Professor of Plant Pathology in the University of Illinois, died August 18, 1934, in his sixty-third year, at Winnetka, Illinois. He had held a preeminent place among mycologists for two decades and had been a prominent, active, and productive personage in American plant pathology for nearly thirty-five years. His premature death ended a life exceptionally useful in his chosen fields and as a teacher, the excellence and sincerity of whose instruction have found expression with gratifying frequency in the achievements of his students. He was highly regarded in America and much honored abroad, and his passing has left a conspicuous gap in the ranks of those devoted to applied and technical mycology.

Descended from a pioneer family of Onondaga County, New York, the environment of his early years, spent on a farm near Syracuse, fostered the development of that inborn love of nature which was, throughout his life, the inspiration of his accomplishments. As a youth, he made a comprehensive collection of the ferns of Onondaga County and also a representative collection

<sup>1</sup> Biographical data are given in both Who's Who in America and American Men of Science. Space does not permit printing the long list of articles and books written by F. L. Stevens.

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of geological specimens from the same region; and he equipped for himself, at home, a laboratory in which, with only the aid of manuals and texts, he obtained such a mastery of chemistry as to be able to pass examinations and to graduate from both high school and college with chemistry courses to his credit, without having had any formal instruction in the subject.

At about the time of his graduation from Hobart College Stevens, while casting about for some phase of natural science to which he could devote himself with a prospect of usefulness and success, went on a visit to the Agricultural Experiment Station at Geneva, New York. There he discussed his problem with David G. Fairchild, who suggested that the field of fungi and plant diseases appeared to offer a splendid opportunity and that he might do well to associate himself for study with Fairchild's uncle, Byron D. Halsted, then pioneering in American plant pathology. Acting on this suggestion Stevens went to Rutgers College, where between 1891 and 1893 he acted as student assistant and in the latter year received the B.S. degree.

Stevens then became teacher of science in Racine College for a year and while there became acquainted with Dr. J. J. Davis, at that time a practicing physician developing the interest in fungi which has since given him a unique position in American mycology. The following year Stevens became teacher of chemistry and botany in the high school at Columbus, Ohio, and it was while holding this position that he received the stimulus which brought him eventually into the broader field of his life's work. Though not formally a student in the University of Ohio, he had been given the privilege of working in the University's laboratories. While satisfying on one occasion his desire to ramble in the woods, he rested on a fallen log and at the moment picked up some leaves attacked by a parasitic fungus. Returning with them to the laboratory, he found them to contain all stages of the developing oöspores of an *Albugo*. The studying of this material began to occupy his time and attention and, after receiving the M.S. degree from Rutgers College in 1897, he went to the University of Chicago where, as fellow in botany, he completed his classical studies of the fertilization and development of the oöspores of *Albugo* and received, for his thesis, the Ph.D. degree, *magna cum laude*, in 1900.

The University of Chicago awarded him a traveling fellowship for the following year and he went first to Bonn and Halle, studying under Strasburger, Klebs, and others, and later to the Naples Zoölogical Laboratories, where he was awarded a table under the auspices of the Smithsonian Institution.

Upon returning from Europe he became, first, instructor in biology, and then professor of botany and plant pathology in the North Carolina College of Agriculture and Mechanic Arts, which position he continued to hold until 1912. During many of these years he was also biologist and head of the department of plant diseases of the North Carolina Agricultural Experiment Station.

The years in North Carolina were noteworthy in Stevens' life for many things subsequently of importance in the science of plant pathology, but the extent of his interest was never limited to pathology alone. It was here that he wrote "Diseases of Economic Plants" with J. G. Hall, the first comprehensive handbook of plant diseases published in America. Here, too, he undertook the study of many problems of fundamental importance in American plant pathology, notable among them control of tobacco diseases by soil sterilization and breeding and selection of cowpeas and watermelons resistant to wilt diseases, and championed the use of the "ose" termination for names of plant diseases. Feeling that school books might be made more vitally interesting to children on farms, he collaborated with others of the college and became the joint author of "Agriculture for Beginners," the "Hill Readers," and a "Practical Arithmetic," books intended to facilitate learning by school children through the use of familiar agricultural facts and illustrations.

In his forty-first year Stevens resigned his position in North Carolina to become Dean of Agriculture in the University of Porto Rico and undertook the organization of the College of Agriculture and Mechanic Arts of that institution. While in Porto Rico his insatiable curiosity caused him to make an extensive collection of Porto Rican fungi, which he brought with him on his return to the United States. This collection became the basis of many important mycological contributions by himself and his students. The manuscript of his best known book, "The Fungi Which Cause Plant Disease," was prepared and sent for publication during his sojourn in Porto Rico.

Upon his return to the United States in 1914 he was appointed Professor of Plant Pathology in the University of Illinois, and this position he maintained until his death. At Illinois, however, his interest gradually turned from plant pathology to mycology, in part because of the development of specialized fields of plant pathology in the Illinois Agricultural College but more particularly because, with his advancing years and increasing maturity, the finely intellectual problems of taxonomic mycology, the importance of which his early associations had emphasized, appealed to him more and more strongly.

While his accomplishments in plant pathology, largely realized before his arrival at the University of Illinois, have given him an outstanding place among early American workers, the full importance of his achievements in mycology are yet to be fully appreciated. As an active collector, he traveled into South America, Panama, Hawaii, and the Philippines, returning from each place with large numbers of originally and personally collected specimens from which he developed pioneer studies of the mycological floras of these regions. These studies and his monographic treatments brought him world-wide renown.

Stevens received many honors at home and abroad. The University of San Marcos, at Lima, conferred upon him in 1925 the Sc.D. degree. On the Illinois campus he was a marked figure in the scholarly lines of professors, when gowns and hoods were in order, because of a brilliant scarlet gown worn in signification of the LL.D. awarded by the University of Glasgow in 1928. He was, during his years in North Carolina, a member of the North Carolina State Board of Examiners for the certification of teachers and, in later years, took much pride in his success as a lecturer at farmers' institutes. He was a Fellow of the American Association for the Advancement of Science, a member of the Wisconsin, Ohio, and Illinois academies of science, president in 1905 of the North Carolina Academy of Science, a member of the American Genetic Association, of the Botanical Society of America, the Society of American Bacteriologists, the American Society of Naturalists, the American Nature Study Society, of which he was vice president in 1908, the American Phytopathological Society, of which he was president in 1910, and the Mycological

Society of America, as well as many others. Since 1910, Stevens' name has been among those starred in American Men of Science.

I find it impossible to close this tribute without a personal word in which all of his students, many of whom are also my friends, may join. He was always unassuming and modest, uniformly courteous and patient, with a tendency to speak little of himself but to be much concerned with the good of others. Yet few, if any, of us ever knew him intimately, for his time and his thoughts were so completely devoted to us, in order that we might get all he had to give, that his own affairs were never in evidence. In a University which gives no degree in plant pathology and in a Department which requires of its students first of all a general proficiency in botany, graduate students interested in all phases of botany came under his instruction, and for years those majoring in his subjects made up the majority of the advanced students in the department. Almost without exception, his students are today holding honorable and influential positions in teaching or research in plant pathology and mycology. Among the 285 charter members of the Mycological Society of America, 10 have been students of his and 8 of these obtained their doctorates under him.

In his teaching he was inspiring and at the same time particular and thorough. While he believed, and sought always to impress his students with the belief, that deliberate efforts to create things of immediate usefulness were almost certain to result in shoddy work and that the project method was the greatest pitfall of research, the mysteries of natural phenomena always presented to him the challenge of a needed solution, and the great variety of subjects that came under his consideration, and to which he endeavored to introduce his students, made association with him stimulating indeed. He made use, not so much of his own knowledge and experience, as of examples and the best of literature. It may be said that in all his teaching he followed the behest of Abel, the mathematician, who explained his own accomplishments briefly by saying he had studied the masters, not the pupils. Himself a master, it was to the inspiration of other masters that Frank Lincoln Stevens sought to lead his pupils.

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# SOME CONIDIAL PHYCOMYCETES DESTRUCTIVE TO TERRICOLOUS AMOEBAE

CHARLES DRECHSLER

(WITH PLATES 1-7)

## INTRODUCTION

In isolating parasites referable to the genera *Pythium*, *Phytophthora* and *Aphanomyces* from decaying plant tissues, I have been employing for some years a method which as described earlier (7) entails no attempt through surface disinfection at avoiding contamination of the isolation plates by the extraneous organisms habitually present on such material. After serving their immediate purpose, the isolation plates, as might be expected, often come to display a varied intermixture of adventitious plant and animal life, the character of the mixture in each case evidently depending somewhat on the nature and in larger measure on the source of the material used. Subterranean parts like roots, tubers, stolons and basal portions of stems, or even aerial parts like fruits and leaves that have been in contact with the ground, generally yield *Amoebae* of different species, the smaller forms apparently feeding mostly on bacteria, the larger ones ingesting cysts of the smaller *Amoebae*, testaceous protozoans, nematode eggs and more especially fungous spores of all descriptions.

While *Amoebae* in agar cultures are thus revealed as very active in the rather indiscriminate destruction of fungous life, they are in turn subject to wholesale destruction here and presumably also in nature by specific parasitic and predacious fungi. In a previous note (9) the morphological features of five fungi predacious on *Amoebae* were set forth, four of these being readily recognizable as Phycomycetes in spite of their Hyphomycete-like aerial conidia and of a vegetative mycelium which in three of the species is comparable in delicacy to the mycelium in species of *Actinomyces*. Several other forms similarly producing non-catenulate conidia and

similarly displaying moderately extended mycelial or haustorial development within the smaller *Amoebae* captured by them, have since been observed. Although the sexual reproductive bodies of one of these additional forms are somewhat larger than those of any of the three for which such bodies were figured earlier (9, figs. 3-5), they are yet of dimensions too small to provide in themselves entirely decisive evidence as to their exact structure; or, for that matter, in view besides of the little differentiated and hence undistinctive character of the fusing elements, as to their correct interpretation. In referring earlier to the ambiguous sexual apparatus in the three delicate predacious Phycomycetes as consisting individually of oögonium, antheridium and oöspore, rather than of gametangia and zygosporangium, consideration was given more particularly to resemblances in general arrangement to the sexual apparatus figured by Arnaudow (1, figs. 4, 5) for *Zoophagus insidians* Somm., a predacious fungus of larger dimensions, whose position among the Oömycetes there has been little reason to question.

These resemblances and the possible taxonomic significance attaching to them are by no means yet to be dismissed. In any event, however, it is believed that the troublesome obscurity in structural detail like the attendant morphological ambiguity may in some measure be dispelled through an examination of several fungi of generally greater dimensions and more obvious distinctiveness, which have been found destructive to some of the larger species of terricolous *Amoebae*. Moreover, the fungi in question are well deserving of study in themselves, presenting remarkable novelties in vegetative development together with noteworthy peculiarities in sexual reproduction. And though the two variations in asexual reproduction would seem, when considered by themselves, rather commonplace both with reference to the shape of the conidia and to the arrangement in which the conidia are formed, when considered as pertaining to the Phycomycetes, they constitute departures as unexpected as they are difficult to homologize with the different types of asexual reproduction recognized in that class. Associated with the diversity in shape of thallus is a diversity in biological habit embracing endoparasitic, ectoparasitic and predacious relationships. Indeed, there are scarcely any distinctive features that are shared by all five species; yet each of the five shows

intimate similarities to one or more of the others, and the several correspondencies interlock in a manner leaving little doubt that one is dealing here with an assortment of naturally related forms.

#### ENDOCOCHLUS ASTEROIDES

Of the endoparasites, the one most frequently encountered on agar plate cultures attacks an *Amoeba* (PLATE 1, A) having a fairly substantial pellicle and finely granular transparent protoplasmic contents. The larger animals when in a more or less rounded condition measure approximately  $60\mu$  in diameter, and exhibit clearly a single prolate elliptical nucleus (PLATE 1, A, n) about  $15\mu$  long and  $10\mu$  wide which contains two darker concavo-convex structures lying close within its periphery, one at each of the poles. A search through the literature has uncovered no description of any *Amoeba* with a nucleus of exactly such structure; though from the frequency of its occurrence on agar plates, the animal would seem to be abundant in soil and in decaying vegetable materials, and therefore could hardly be supposed not to have been encountered previously by protozoologists. Undoubtedly the animal comes within the scope of Penard's (15, p. 83) statement: "Il me parait donc préférable—de considérer comme *A. terricola* toutes les amibes terrestres, revêtues d'une pellicule, et pourvues d'un noyan unique et toujours ellipsoïdal." It will accordingly be designated here by Greeff's binomial, together, when necessary for more specific reference, with a numeral: *Amoeba terricola* I.

Contact between host and parasite is established as the animal moves along on the surface of the substratum on which conidia of the fungus are distributed; the distribution showing usually a noticeably linear arrangement wherein the positions of the aerial sporiferous hyphae, now evacuated and disintegrating, remain recognizable. The conidia thus encountered adhere to the pellicle of the host with sufficient firmness to withstand the jostling incident to continuing locomotion. Germination follows, a somewhat bulbous body being first produced, which apparently functions as an appressorium. From this bulbous part is thrust forth a tube of lesser diameter that perforates the pellicle and extends a distance of about  $10\mu$  into the protoplasmic interior of the animal (PLATE 1, A, a). The tip of this tube now swells up into a globular

body which increases in size with the gradual transfer to it of all the material in the conidium (PLATE 1, A, b). When the transfer is accomplished, the globular body soon becomes disarticulated from the germ tube as the result of slight strains incident to the movements of the animal. The evacuated germ tube is then usually promptly expelled by the animal, but the globular body distinguished by the presence of a noticeable apiculum where it was earlier attached is retained within (PLATE 1, A, c, c) to be moved and jostled about passively in the protoplasmic streams together with the nucleus and often also with other inclusions like ingested protozoan cysts, testaceous rhizopods, and miscellaneous fungous spores in various stages of digestion. Sometimes the conidium, instead of remaining external to the *Amoeba*, is ingested either previous to germination or after development has begun, so that structures like that represented in plate 1, A, a may be observed being carried about in the protoplasmic streams. Even in these cases the evacuated membrane of conidium and germ tube apparently becomes separated from the globular body and is expelled, since instances of such a membrane remaining attached to thalli of larger sizes have never been seen in material of this species.

The number of infections to an animal depends obviously on the quantity of conidia encountered, which in turn stands in direct relation to the abundance of matured conidiophores. Early in the course of an epizoötic within a petri dish culture, an infected animal usually contains a single parasitic thallus, cases in which two or three thalli are visible occurring only now and then. As the conidia when produced are spaced at intervals approximately equal to the diameter of the *Amoeba*, it is apparent that a single infection would ordinarily result from the passage of an animal over a matured conidiophore at approximately a right angle, while two or three infections might well eventuate from passage at an oblique angle. Later when the epizoötic is well under way and conidiiferous hyphae span restricted areas here and there in an open arachnoid pattern, larger numbers of infections become more usual, so that animals carrying as many as a dozen thalli may be found. When such larger numbers of parasitic thalli are present, they may often be divided according to size into two or three categories, suggesting as in plate 1, A that the infections occurred in

several successive installments corresponding to successive passages of the animal over terrain strewn with conidia.

Vegetative growth of the young globular thallus takes place through widening and elongation at the distal end, at first apparently extending the original axis in a straight line (PLATE 1, A, d, e). When a length of approximately  $10\ \mu$  has been attained, a rather abrupt curvature in direction intervenes, resulting first in an obese U-shaped structure (PLATE 1, A, f, g, h, i). With continued elongation at a more moderate, rather uniform degree of curvature and at a tolerably uniform diameter, modified sometimes by dichotomous branching at one or two points, a helicoid structure of striking appearance is brought into being (PLATE 1, B, C; PLATE 2, A, B). The ultimate size of this structure is conditioned naturally by the size of the animal as well as by the number of other thalli competing with it for the nourishment which the protoplasm of the host affords.

For a considerable period after infection the *Amoeba* shows virtually no evidence of ill effects from the presence of the fungus. The animal shown in plate 1, A, for example, although burdened with four thalli of more than negligible bulk in addition to five smaller ones, was at the time it remained under observation still moving about with apparently undiminished briskness. As the protoplasmic materials become more and more depleted, locomotion becomes gradually slower, the infected *Amoeba* at the same time evidently contracting in volume; yet even in decidedly advanced stages of debilitation (PLATE 2, A) when the bulk of the host has been reduced by perhaps more than a half and the cumbersome mass of fungous thalli on occasion visibly interferes with the free movement of the pellicle, some locomotion may be observed. Finally, of course, movement ceases, and the remaining protoplasmic host materials are absorbed, leaving virtually nothing of the animal except the collapsing pellicle (PLATE 2, B). With exhaustion of its food supply the fungus necessarily terminates its vegetative growth, and devotes its substance to reproduction, either sexual or asexual, or both.

In asexual reproduction the vegetative thallus gives rise usually to a single delicate filament, which, after perforating the enveloping pellicle, forces its way through the overlying substratum to

emerge in the air (PLATE 1, b, c). Aerial growth is continued until the protoplasmic contents of the fungous body have been used up, a length of 1.5 to 2 mm. being usually attained with fairly well developed specimens, while the largest thalli may give rise to aerial filaments twice as long. Sometimes a filament may give off one or more branches, usually near the point of egress from the substratum (PLATE 2, c). Branching however is never abundant, and in any case entails no increase in total length of filament produced.

Once the delicate aerial filament has attained its definitive length septa make their appearance in it at intervals usually of about 50 or  $60\mu$ . Thereupon, mostly in positions approximately median between these septa, protrusions bud forth laterally (PLATE 2, g, a) and increase in size as the contents of the parent hyphal segments migrate into them (PLATE 2, II; g, b; i). The progressive evacuation of the segments is accompanied by the laying down of successive septa in the same manner as in aging mycelium of many species of *Pythium* and *Phytophthora*. When, therefore, the aerial filament becomes entirely evacuated it reveals cross-walls at intervals of from 5 to  $30\mu$ , and bears at distances of 30 to  $80\mu$  the individual conidia into which the protuberances have in the meantime developed (PLATE 1, c). The completed conidium is set off by a basal septum in a plane parallel with and usually tangent to the parent filament (PLATE 2, j, b, c), though in many instances the septum may be inserted a short distance from the axial hypha, so that the spore instead of always being accurately sessile is sometimes borne on a perceptible spur (PLATE 2, j, a, d, e, f). During maturation the protoplasm is withdrawn from the attenuated tip of the conidium, and the evacuated part, after being set off by a wall, persists as a small apical appendage (PLATE 2, j, a-f). Exclusive of this appendage the spore consists of a single fusiform colorless cell, surrounded by a thin smooth wall and filled with finely granular protoplasm. Under low or medium magnification, in undisturbed material, the slightly bristling and markedly linear arrangement of the conidia on the empty parent filaments, now matted down or loosely draped on the substratum, or often suspended like gossamer threads between projecting bits of solid material, provides a distinctive view. Such a view, however, is not

frequently afforded when cultures are infested with energetic creatures like mites, springtails, nematodes or small earthworms, for not only do the conidia become very readily detached from the parent filaments, but the parent filaments themselves become readily broken up even while the conidia are still in course of development.

Sexual reproduction of the fungus is initiated by the proliferation of hyphal outgrowths about twice as wide as those concerned with asexual reproduction. The outgrowths perforate the loosely enveloping host pellicle, and after growing out into the substratum a variable but usually very short distance, make apical or approximately apical contact with one another in pairs. Each pair of hyphae fuse at the place of contact, after which a spherical body develops, sometimes from the junction (PLATE 2, c, d), but much more frequently on a short extension arising from the junction or prolonging one of the hyphae (PLATE 1, b; PLATE 2, c, a, b, c, e, g; f). This body continues to increase in size with the movement into it of protoplasm from below until its definitive dimensions are attained, when it becomes delimited usually by a single basal septum (PLATE 2, c). The protoplast within the spherical cell thus formed then undergoes slow reorganization, developing a rather thick wall of its own (PLATE 2, d, a-d), which in time becomes contracted so as to present a stellate outer profile with six, seven or eight apices (PLATE 2, e, a-h). Between these apices the originally spherical enveloping membrane usually collapses slightly, resulting in relaxed, flat or even depressed facets. The living material occupying the spherical locule within the colorless or slightly yellowish stellate endogenous structure, shows at maturity a parietal layer of rather uniformly coarse granules surrounding a homogeneous central vacuole.

Although the structural relationships of the sexual apparatus are thus tolerably evident, some difficulties of interpretation remain. The very obvious, pronounced separation of the stellate structure from the membrane within which it is formed, is highly suggestive of the separation of oögonial wall and oöspore usual in such monosporous genera as *Pythium* and *Aphanomyces*. In a general way, too, the organization of the protoplasm would seem to conform to the organization usual in oöspores, though the stellate sculpturing injects optical difficulties that make it impossible to determine def-

initely whether or not a refringent body, the "helle Fleck" of de Bary, is present in the parietal granular layer. On the other hand, the relationships of the sexual hyphae to one another and to their joint product are certainly not those ordinarily obtaining between oögonium, antheridium and oöspore, but correspond essentially to relationships known among some Zygomycetes, such as those figured, for example, by Thaxter for his *Empusa echinospora* (16, pl. 19, fig. 298-302) and by Van Tieghem for *Syncephalis Cornu* Van Tiegh. & Le Monn. (18, fig. 88-93). From the account of the latter author, it would appear besides that *S. Cornu* presents similarity to the *Amoeba* parasite also in the development of a spiny spore within the spherical cell arising directly from the fusion of sexual elements, and in the presence of a large "oil drop" in the mature sexual spore. As in accordance with the very plausible "théorie dualiste" referred to by Lendner (12) the sexual spore proper of the Zygomycetes, no less than that of the Oömycetes, is essentially endogenous in origin, the development of the sexual apparatus in the present fungus would seem to require interpretation of the spherical cell as a zygosporangium, and of the stellate structure as a zygospore.

Not infrequently while the zygosporangium is still in course of enlargement, a septum appears in one of the sexual hyphae somewhere between its origin and its juncture with the other hypha. It seems at least questionable whether any special significance can be read into this septation, which in its inconstancy invites comparison with the occasional septation occurring in conjugating hyphal bodies of species of *Empusa*. There is little reason for believing that a septum in one of the sexual hyphae is different in function from a septum in the vegetative thallus itself, where a partition would seem to serve a purpose no more abstruse than the separation of a relatively large mass of protoplasm into portions destined for the production of separate reproductive units. In any case cross-walls are more wont to appear in the larger thalli that participate in the production of several zygosporangia besides giving rise, perhaps, to a conidiiferous filament, than in thalli so small that they become exhausted by participation in the development of a single zygosporangium or by proliferation of a single conidiiferous hypha.

To the extent to which the individual thalli can be distinguished from one another in the more or less intricate tangle into which they are usually compacted through the movements of their animal host, two conjugating hyphae seem regularly to originate from different thalli. Conjugation between filaments from thalli in separate *Amoebae* has never been observed, but presumably might well occur if two infected animals should die about at the same time and in close proximity to one another. Apart from the exigencies of a behavior strongly suggestive of heterothallism, the type of reproduction, whether sexual or asexual, is influenced appreciably by proximity to the air. When an infected *Amoeba* containing a number of thalli succumbs on or near the surface of the substratum, a more abundant development of conidia relative to zygosporangia ordinarily ensues than when the animal dies at a greater depth in the substratum. In the end, whatever the type of reproduction, the evacuated thallus membranes together with the pellicle of the host shrink into an inconspicuous wrinkled mass in which the separate constituents are indistinguishably confused (PLATE 2, D, E).

The parasite is apparently not eligible for inclusion in any genus of the Zygomycetes hitherto described. It would seem to represent the type of a new genus for which a name having reference to its curious spiral endozoic vegetative habit may be appropriate.

#### *Endocochlus* gen. nov.

Hyphae nutritiae intra corpus amoebarum viventium evolutae, primo continuae, breviusculae, latae, simplices vel parum dichotomae, in spiras convolutae, animali emortuo extus hyphas conidiferas et hyphas zygosporiferas emittentes. Conidia aeria, hyalina, fusoidea vel ellipsoidea, hinc inde ex hyphis acriis repentinibus tenuibus arachnoideis oriunda assurgentia. Zygosporangia globosa intra materiam animal emortuum ambiens ex apice hypharum duarum similium conjugantium evoluta.

Vegetative hyphae endozoic, stout, simple or sparingly branched, when well developed often rather regularly and compactly convoluted, at first non-septate, after death of animal giving rise to conidiiferous and zygomorphic hyphae. Conidia aerial, hyaline, fusoid or elliptical, borne singly at intervals on long aerial hyphae. Zygosporangia globose, produced in substratum outside of animal host at junction of two similar conjugating branches or on a short hyphal extension from such junction.

**Endocochlus asteroides** sp. nov.

Hyphae nutritae 4.5–8  $\mu$  diam., semel vel bis spiraliter convolutae. Conidia fusoidea, 11–19  $\times$  3.2–4  $\mu$ , in apice appendicula 1.5–5  $\mu$  longa praedita, ex hyphis arachnoideis 1.2–1.4  $\mu$  crassis 1–4 mm. longis ad intervalla 30–80  $\mu$  longa enata. Zygosporae hyalinæ vel luteolæ, echinatae, intra zygosporangium sphaeroideum 11–14  $\mu$  diam. formatae, loculo 5–7.5  $\mu$  diam. Hyphae zygosporiferae 2.3–4  $\mu$  crassæ, 30–65  $\mu$  longæ.

Hab. in *Amoeba terricola* Greeff (in senso lato), Washington, D. C.

Vegetative hyphae 4.5 to 8  $\mu$  in diameter, simple or often when well developed sparingly branched dichotomously and compactly convoluted in 1 to 2 turns. Conidia spindle-shaped, measuring 11 to 19  $\mu$  (mostly 12 to 16  $\mu$ ) in length by 3.2 to 4  $\mu$  (average about 3.6  $\mu$ ) in diameter, exclusive of an empty apical appendage 1.5 to 5  $\mu$  (mostly 1.5 to 3  $\mu$ ) long and about 1  $\mu$  wide at base; produced more or less erect and sessile or nearly sessile at intervals of 30 to 80  $\mu$  (mostly 50 to 60  $\mu$ ) on aerial hyphae 1 to 4 mm. long and 1.2 to 1.4  $\mu$  wide. Zygospore colorless or slightly yellowish; at maturity broadly echinulate, the rather thick wall provided with about 20 protuberances of which 6 to 8 are visible in the stellate profile; containing a locule 5 to 7.5  $\mu$  in diameter; produced within a spherical zygosporangium 11 to 14  $\mu$  in diameter which arises mostly from a short hyphal extension from the junction of zygomorphic hyphae that measure usually 30 to 65  $\mu$  in length and 2.3 to 4  $\mu$  in width.

Destructive to *Amoeba terricola* (in the broad sense more particularly of Penard) in cultures prepared from decaying roots of various herbaceous plants collected near Washington, D. C.

**COCHLONEMA VERRUCOSUM**

In a single agar plate culture a species of *Amoeba*, somewhat smaller than *A. terricola* I, was found parasitized by a fungus having much the vegetative habit of *Endocochlus asteroides*, but differing from it especially in asexual reproduction. When in a moderately rounded condition the larger individuals of the species of *Amoeba* attacked measured between 50 and 60  $\mu$  in diameter. This dimension, considered together with the slightly elliptical or nearly spherical shape of the nucleus (PLATE 3, E), the finely granular transparent character of the protoplasm and the delicate yet firm pellicle, permitted fairly plausible identification of the animal as *A. sphaeronucleolus* Greeff.

At the time the epizoötic was discovered it evidently had already progressed well towards its end, as all the individuals of the susceptible species still alive in the culture bore within themselves thalli of the parasite, varying in number from one to three, at different stages of development (PLATE 3, D, E). Consequently the entrance of the fungus into the animal could not be directly observed, a circumstance of lesser moment, however, as the mode of ingress could here be inferred even from material in advanced stages of development. For very generally to each of the endozoic thalli was still attached a small fusoid body with minutely verrucose membrane, readily recognizable as the conidium from which growth had proceeded (PLATE 3, C, D, E). Manifestly the conidium, after having been ingested by the hapless animal, had germinated laterally, the germ tube immediately widening out and directing its growth spirally, so as to yield a convolute thallus slightly less stout but otherwise resembling that of *Endocochlus asterooides*.

Asexual reproduction of the fungus is initiated when the contents of the animal have been reduced to such an extent that locomotion has virtually ceased (PLATE 3, D), though nucleus, contractile vacuole and remnants of cytoplasm usually continue for some time to present a fairly normal appearance (PLATE 3, E). Mostly from a position near the proximal end of the thallus is put forth a delicate hypha which perforates the pellicle and, if the animal is at all submerged, makes its way to the surface of the substratum. Near or on this surface, if it has not already become branched, the filament undergoes some ramification (PLATE 3, C, E; PLATE 4, A). The resulting branches after spreading in divergent directions for variable distances, grow up into the air, where each gives rise to a more or less erect aerial chain of usually 30 to 40 fusoid verrucose conidia (PLATE 4, A).

The chains of conidia show the general features associated with the genus *Fusidium* in the Hyphomycetes. In spite of the considerable attention devoted to the details of spore formation in this species as well as in the other three catenate forms to be described, it has not been possible, owing to difficulties attending observation, to determine this phase of developmental morphology with as much certainty as might be desired. The structures concerned are too small to be studied successfully in their normal

aerial state under a dry objective. On the other hand when developing and hence still immature sporogenous filaments are mounted in water, their appearance suggests so strongly a prompt intervention of degenerative change that the normal course of events remains somewhat a matter of inference. However, judging from the more satisfactory microscopic preparations, it would seem that the spore chains have origin in continuous aerial filaments which from an early stage, if, indeed, not from the very beginning, are characterized by *Leptomitus*-like constrictions spaced at equal intervals (PLATE 3, E; PLATE 4, A, b, c). These regularly constricted filaments exhibit minute verrucose sculpturing that does not extend proximally into the sterile parts of the supporting hyphae. Apparently some slight increase in size takes place before the distended parts become separated from one another through withdrawal of protoplasm from the constricted isthmi and formation of a delimiting partition at either end of the individual swellings (PLATE 4, A, a, d). In a mature condition the chains are very easily broken up, so that the conidia soon come to lie separately on the surface of the substratum (PLATE 3, F), awaiting the passage of a susceptible animal, and a repetition of the developmental cycle just outlined.

Sexual reproduction of the fungus was observed in connection with but a single animal, and in this was represented by only two unions (PLATE 3, G). Unfortunately, moreover, the membranes of the empty thalli and their hyphal outgrowths were already so badly collapsed that none of the zygomata could with certainty be followed backward any considerable distance from junction to origin. The junction, which in both cases was revealed plainly, appeared similar to that in *Endocochlus asterooides*, and as usually in the latter fungus bore the zygosporangium on a short prolongation. The zygosporangium here, however, was much more distinctly yellowish in coloration, and instead of being smooth, bore numerous prominent bullate protuberances. Within this sculptured primary fusion cell, to extend the contrast, was revealed in the one nearly mature specimen a zygospore with a smooth wall of moderate thickness.

In shape and sculpturing of conidium as well as in the persistence of that structure on the endozoic thallus, the fungus shows

obvious similarities to the form which Penard (15) found parasitizing *Amoeba alba* Greeff and discussed as *Saprolegnia* B. Although the Swiss zoölogist did not succeed in observing the production of the conidia described by him, very probably because he employed mostly cultures of aquatic rather than terrestrial character, their close correspondence to those of the present fungus gives much ground for believing that he may have been dealing with an intimately related catenulate form. It may be mentioned that a cochleate endoparasite with catenulate conidia of approximately the large dimensions given by Penard occurred some years ago on an unidentified *Amoeba* in one of my agar plate cultures, though because of unfamiliarity with the type of fungus in question, which was then mistakenly judged to be referable to *Fusidium*, the scanty material was dissipated before more accurate records had been made of morphological details. However, the method of reproduction by zoöspores that was attributed, even if only with partial assurance, to *Saprolegnia* B, has never been observed either in the inadequately studied species or in any of the catenulate forms dealt with herein; nor would the swollen rather irregularly branching mycelium figured by Penard appear to conform any too well to the regularly involute type of endozoic thallus.

Since the mode of asexual reproduction represented here differs conspicuously from that set forth as characteristic of *Endocochlus*, a separate genus is proposed:

#### *Cochlonema* gen. nov.

Hyphae nutritae intra corpus amoebarum viventium evolutae, primo continuæ, breviusculæ, latae, simplices vel parum ramosæ, in spiras convolutæ, animali emortuo vel moriente extus hyphas conidiferas et hyphas zygosporiferas emitentes. Conidia aria, hyalina, fusoidea vel elongata, in catenulas longiusculas simplices plus minusve erectas digesta. Zygosporangia globosa, intra materiam animal emortuum ambiens ex apice hypharum duarum similiūm conjugantium evoluta.

Vegetative hyphae endozoic, stout, simple or sparingly branched, when well developed often rather regularly and compactly convoluted, at first continuous, with decline or on death of host animal giving rise to delicate conidiiferous and usually somewhat stouter zygomorphic filaments that pass through the host envelope. Conidia aerial, hyaline, fusoid or elongated, produced in long

chains from more or less erect aerial hyphae. Zygosporangium globose, produced on a short prolongation from the apical junction, or directly from the junction of two similar filaments, in the material surrounding or underlying the animal.

**Cochlonema verrucosum** sp. nov.

Hyphae nutritae 4.5-7  $\mu$  diam., semel vel bis spiraliter convolutae. Conidia verruculosa, fusoidea, 6-9  $\times$  1.4-2  $\mu$ , ex hyphis saepius 1.2-1.5  $\mu$  crassis enata, in quaque catenula tricena usque quadragena. Zygosporangia flavidia, globosa, circa 12  $\mu$  diam., 20-30 verrucis ornata; verrucae 1.5-2  $\mu$  altae, basi 2.5-3  $\mu$  diam.; zygosporae globosae. Hyphae zygosporiferae breviusculae, 2-3  $\mu$  crassae.

Hab. in *Amoeba sphaeronucleolo*, Washington, D. C.

Vegetative hyphae usually 4.5 to 7  $\mu$  in diameter, simple or often when well developed sparingly branched and rather compactly convoluted in 1 to 2 turns. Conidia minutely verrucose, fusoid, measuring 6 to 9  $\mu$  in length by 1.4 to 2  $\mu$  in diameter, produced in chains usually of 30 or 40, on hyphae mostly 1.2 to 1.5  $\mu$  wide. Zygosporangia yellowish, globose, measuring about 12  $\mu$  in diameter exclusive of the warty protuberances 20 to 30 in number that measure often 2.5 to 3  $\mu$  in basal diameter and 1.5 to 2  $\mu$  in height; zygosporae smooth, globose; zygomorphic hyphae rather short, mostly 2 to 3  $\mu$  wide.

Destructive to *Amoeba sphaeronucleolus* in a laboratory culture, Washington, D. C.

**COCHLONEMA DOLICHOZPORUM**

A fungus very similar to the one just described was found in small quantity in another agar plate culture destroying *Amoebae* having approximately the same dimensions as *Amoeba sphaeronucleolus*. Animals in stages of infection early enough to reveal their nuclear condition were not observed, however, so that the identity of the host remains for the time being uncertain. As in the case of *Cochlonema verrucosum* the conidium here is evidently ingested bodily, since it likewise is regularly to be seen attached to the endozoic thallus, though attached somewhat less closely by a delicate germ tube of appreciable length (PLATE 3, A; PLATE 4, B). Asexual reproduction results here also in chains of aerial conidia (PLATE 4, B, a, b, c), the individual conidium being, however, two or three times as long, somewhat more prominently sculptured, and often when fully mature evacuated of protoplasm

in the distal narrower part, which then nevertheless persists as an empty appendage (PLATE 3, b). Accordingly, whereas the conidium when newly delimited is ordinarily slightly obclavate in shape, after evacuation of the appendage the cell left filled with protoplasm is more nearly symmetrical, that is, roughly elongated fusoid in shape. Sexual reproduction has not been observed.

**Cochlonema dolichosporum** sp. nov.

Hyphae nutritae circa  $6\mu$  diam., scemel vel bis spiraliter convolutae. Conidia verrucosa,  $15-25 \times 1.2-2\mu$ , ex hyphis 1-1.5 crassis enata, in quaque catenula quina usque vicena, primo saepius nonnihil obclavata, maturitate apice saepius vacuo, tum cellula viventi plus minusve fusoidea vel cylindracea. Zygosporae ignotae.

Hab. in *Amoeba* sp., Washington, D. C.

Vegetative hyphae approximately  $6\mu$  in diameter, convolved into a spiral of 1 to 2 turns. Conidia verrucose, measuring 15 to  $25\mu$  in length and 1.2 to  $2\mu$  in width, borne in chains of 5 to 20 each on hyphae 1 to  $1.5\mu$  wide, at first somewhat broader in proximal than in distal portion, later often becoming evacuated in the narrower apical part, which then persists as an appendage up to  $7\mu$  long on the cylindrical or somewhat fusoid living cell. Zygospores unknown.

Destructive to *Amoeba* sp. in a laboratory culture, Washington, D. C.

BDELLOSPORA HELICOIDES

A fungus strongly resembling the two species of *Cochlonema* in its asexual reproduction was observed in a number of agar plate cultures, usually in quantity, habitually parasitizing a species of *Amoeba* the larger individuals of which when fairly well rounded up measured from 60 to  $90\mu$  in diameter. The cytoplasm of the animal was in general finely granular, colorless and decidedly transparent; its pellicle firm, perhaps rather thicker than in most terrestrial forms, and therefore inclined to be cast into fewer but more pronounced folds. The single nucleus (PLATE 5, b, n) having the shape of a prolate ellipsoid of revolution showed at the poles accumulations of darkish irregular lumps that thinned out to a single layer in the equatorial periphery. The structural features of the animal thus correspond tolerably well to those that Penard (15) ascribed to his *A. terricola* var. *papyracea*. Yet no

close approximation to the considerably larger dimensions cited by Penard has ever been observed in my material, so that a presumption of identity would seem at least doubtful. The animal, of course, readily conforms to Penard's broad concept of *A. terricola*, and may thus be conveniently referred to as *A. terricola* II, the appended numeral being intended to distinguish it from the habitual host of *Endocochlus asterooides*, as well as from the *Amoeba* attacked by the fungus to be described as *Zoopage phanera*.

Infection is initiated in much the same way as was described in the account of *Endocochlus asterooides*. Conidia of the parasite strewn about on the surface of the substratum (PLATE 5, A) make contact with the *Amoeba* as it passes over them and remains adhering to the pellicle in spite of subsequent locomotion of the animal both on and through the substratum. Each adhering spore soon puts forth a delicate germ tube which perforates the pellicle and continues in its course into the host for a distance often about equal to the length of the conidium (PLATE 5, B, a). From this stage on, analogy with *E. asterooides* ceases. The tip of the infective germ tube instead of developing a globular body into which the conidial contents are received, becomes dichotomously branched (PLATE 5, B, b-f). Each of the divergent limbs soon branches in its turn, and in a plane at a right angle to that of the original dichotomy (PLATE 5, B, g). A third bifurcation follows the second (PLATE 5, B, h), and in some cases a fourth bifurcation takes place (PLATE 5, B, i), so that eventually a compactly branching apparatus with 8 to 16 terminal elements is brought into being. This apparatus has all the appearance of a haustorium and, indeed, manifestly is one; for coincident with the first bifurcation the spore outside of the animal begins to show perceptible swelling (PLATE 5, B, a-f). This swelling continues until the conidium has expanded into an obese ellipsoidal body whose mode of origin is later often betrayed only in two minute protruding polar apiculi representing the apparently inelastic spore extremities (PLATE 5, B, i).

While in the early stages of an epizootic individual *Amoebae* are often found with but a single infection, plural infections become the rule subsequently, when with an increasingly abundant

and thoroughgoing distribution of conidia the encounters between host and parasite become more and more frequent. Accordingly in the more advanced phases of an epizoötic, animals beset with ten or a dozen separate plants in various stages of development are not of infrequent occurrence (PLATE 5, B). Locomotion although impeded physically by the presence of the larger swollen spore bodies, usually continues for some time after the progressive exhaustion of protoplasmic contents has entailed a readily noticeable contraction of the animal's bulk. Its cessation, in fact, ordinarily is brought about not primarily by exhaustion of the host as in the case of *Amoebae* infected with *Endocochlus asteroides*, but through virtual anchoring of the host to or in the substratum that takes place with the proliferation of hyphal outgrowths from spore bodies preparatory to reproduction. The anchored animal continues to furnish food materials to the parasite during a protracted period of reproductive development, so that conidial chains (PLATE 6) or sexual apparatus (PLATE 5, C) may be displayed in abundance even while the host animal is still alive. The parasitized *Amoeba* in the last stages preceding death is rounded up into an almost spherical shape, its pellicle showing few irregularities except for the depressions where the spore bodies are attached (PLATE 5, C; PLATE 6). Even with the animal reduced to perhaps a third of its original bulk, the granular material now remaining suffices only to provide a thin parietal layer surrounding an immense central vacuole. Often protruding conspicuously from the parietal cytoplasmic layer into the central vacuole is a vesicle-like vacuole, evidently to be interpreted as the contracting vacuole, now greatly enlarged apparently owing to its incapacity to discharge through the thickened pellicle.

As has been intimated asexual reproduction of the ectoparasite follows the same general course as in *Cochlonema verrucosum* and *C. dolichosporum*. Like the convolute endozoic thalli of these fungi, the swollen spore bodies here put forth a single hypha, or less often several hyphae. Each filament, after traversing the substratum for a variable distance depending in some measure at least on the depth at which the host animal was halted, reaches the surface where immediately or after some prostrate growth its further extension becomes aerial and more or less erect. One or

several branches may be given off in either the intramatrical (PLATE 6, c), the superficial (PLATE 6, b), or the aerial (PLATE 6, a-d) part. In any case, on each of the resulting aerial hyphae is formed terminally a chain (PLATE 6, a, c; b, d; c, a, b; d) of irregularly fusiform conidia, between a half-dozen and a score in number. With an adequate supply of nourishment development of a second chain of spores from a branch having origin just below the base of the first chain (PLATE 6, a, b, d; b, a, c), and even of a third chain from a second branch, may ensue. On maturity the chains are broken up on relatively slight disturbance, as, for example, on being brushed by passing nematodes; so that the conidia are soon strewn about on the substratum, ready to infect any susceptible *Amoeba* that may happen to come along.

The external position of the spore bodies on the animal, and their globose shape that makes it easy to distinguish them from one another even under crowded conditions, provide in this species circumstances more favorable for determining the relationships involved in sexual reproduction than obtain in any of the related convoluted endozoic parasites. In spite of a frequently somewhat intricate arrangement here of the zygomorphic hyphae themselves, two conjugating filaments can always be traced back to separate spore bodies, never to the same spore body (PLATE 5, c). These filaments whether representing direct outgrowths of their respective spore bodies (PLATE 5, c, d), or primary (PLATE 5, c, c) or even secondary branches of such outgrowths (PLATE 5, c, a, b), usually engage one another very close to the swollen structures, if not in immediate proximity to them. After establishing apical contact they continue to elongate, twisting about one another in conspicuously regular close helical turns. Each of the hyphae makes from two to four turns, the total number of windings in the intertwined helices thus varying usually between four and eight. No discrimination with respect to direction of rotation, whether dextrorse or sinistrorse, is evident.

The interwoven hyphae now fuse apically or approximately apically. On the end of a short cylindrical part continuing one of the hyphae or arising more symmetrically from the junction, a globose body buds out and increases in size to form a zygosporangium (PLATE 5, c). By this time a septum is present in each of

the zygomorphes usually at a point about median in the intervolved part. The distal halves of the zygomorphic filaments delimited proximally by these septa constitute elements that would seem to correspond more plausibly to the gametangia of the more familiar genera in the Zygomycetes than any structural constituents of the sexual apparatus produced by any of the other fungi herein described, or by any of the minute predaceous forms figured earlier (9, figs. 3-5). After the terminal zygomorphic parts have contributed their contents to the zygosporangium, the latter is partitioned off from its supporting element by a basal septum. In the meantime the originally smooth zygosporangium has become sculptured through the putting forth of noticeably thick-walled wart-like or bullate protuberances. Internal development now ensues with the result that finally the sculptured wall comes to envelop rather closely a zygospore proper, which at maturity has a smooth wall of moderate thickness, and within this wall a parietal layer of uniformly and rather coarsely granular material surrounding an apparently homogeneous, relative large reserve globule (PLATE 5, d-h, i, a-h). A clustered arrangement of these distinctly yellowish sexual structures in the substratum about a wrinkled residual mass in which the shrunken pellicle of the host and the collapsed membranes of the parasite are mostly unrecognizably confused, remains to mark the place where an animal came to its end (PLATE 5, i). Indeed, just as in the case of *Endocochlus asteroides*, the presence of such clustered arrangements often constitutes the only testimony to a former abundance of the susceptible *Amoeba* species in cultures from which it has been exterminated.

The close resemblance of the zygosporangium and zygospore to those of *Cochlonema verrucosum* suggests the possibility that the remarkable intervolution of zygomorphes in the present fungus, so reminiscent of the relationships of *Syncephalis nodosa* van Tiegh. as illustrated in the beautiful figures of Bainier (2, 3) and of Thaxter (17), may constitute a feature having significance only as a character pertaining to the species. A consideration of sexual as of asexual reproduction, therefore, reveals no decisive reason for not assigning the fungus to *Cochlonema*. However, the remarkable epizoic habit with its striking analogy to the habit

prevalent among the Rhizidiaceae in the Chytridiales, offers so direct an antithesis to that exemplified in *Cochlonema* and *Endocochlus* that assignment to either of these genera would seem inappropriate. A separate genus is accordingly proposed under a name which is intended to bring into relief the leech-like behavior, so to speak, of the conidium.

**Bdellospora** gen. nov.

Conidia aeria, hyalina, fusoidea vel elongata, in catenulas longiusculas simplices plus minusve erectas digesta; ad pelliculam animalium adhaerentia, hypha germinationis pelliculam perforantia, haustorium ramosum intus evolventia, tum magnopere tumescientia, mox hyphas conidiferas et hyphas zygosporiferas emitentia. Zygosporangia globosa intra materiam animal emortuum ambiens ex apice hypharum duarum similiūm conjugantium evoluta.

Conidia aerial, hyaline, fusoid or elongate, arising in frequently long chains from more or less erect aerial hyphae; adhering to animals and after individually perforating the pellicle or integument by means of a germ tube that develops into a haustorium inside, swelling into large globose-ellipsoidal bodies from which conidiiferous and zygomorphic hyphae grow out. Zygosporangia globose, developed in the material surrounding or underlying the dying animal from the junction of two similar conjugating filaments.

**Bdellospora helicoides** sp. nov.

Conidia fusoidea, nonnihil angulata,  $6-16 \times 2-3 \mu$ , ex hyphis  $1.3-2 \mu$  crassis enata, in quaque catenula quina usque vicena; post amplificationem globoso-ellipsoidea, usque  $15 \mu$  diam.,  $20 \mu$  longa, haustorio pedicellato usque ter vel quater breviter bifurcato. Hyphae zygosporiferae inter se quater vel octies spiraliter circumPLICANTES, utraque ex conidio turgido alio enata, basi saepius  $2-2.5 \mu$  crassae, sursum  $3-4.5 \mu$  crassae,  $30-75 \mu$  longae, septo in partes duas sere subaequaliter divisae. Zygosporangia  $8-13 \mu$  diam., flavida,  $10-30$  verrucis  $1-1.5 \mu$  altis,  $1.5-3 \mu$  diam. ornata. Zygosporae globosae.

Hab. in *Amoeba terricola* (in senso lato), Washington, D. C.

Conidia fusoid, measuring 6 to  $16 \mu$  (average  $10 \mu$ ) in length by  $2$  to  $3 \mu$  (average  $2.7 \mu$ ) in width, somewhat angular in outline, produced in chains usually of 5 to 20 on aerial hyphae mostly  $1.3$  to  $2 \mu$  wide; after vegetative enlargement globose-ellipsoidal, measuring up to  $15 \mu$  or more in transverse diameter, and up to  $20 \mu$  or more in length, each conidial body being provided with a haustorium consisting of a germ pedicel  $3$  to  $7 \mu$  long and up to  $1.5 \mu$  wide together with short elements up to  $2.5 \mu$  wide

in a terminal closely dichotomous branching system. Zygophoric hyphae 4 to 8 times intervoluted, each of a conjugating pair arising from a separate swollen conidium, measuring 2 to  $2.5\mu$  in diameter at the base but widening toward the apex to a diameter of 3 to  $4.5\mu$ , mostly 30 to  $75\mu$  long, and regularly divided by a transverse septum into 2 nearly equal parts. Zygosporangia mostly 8 to  $13\mu$  in diameter, distinctly yellowish, ornamented with 10 to 30 (mostly about 25) wartlike protuberances measuring 1 to  $1.5\mu$  in height and 1.5 to  $3\mu$  in basal diameter. Zyospores globose, smooth.

Parasitic on *Amoeba terricola* (in the broad sense especially of Penard) in laboratory cultures made from decaying rootlets collected near Washington, D. C.

#### ZOOPAGE PHANERA

More readily visible in its results than any of the parasitic relationships herein discussed is a predacious relationship involving as prey a large and relatively opaque species of *Amoeba* that often becomes very abundant in aging plate cultures. Though the species is somewhat larger even than the one attacked by *Bdellospora helicoides*, measuring between 35 and  $110\mu$  in diameter when drawn up into a more or less rounded shape, its pellicle is so delicate as to appear under high magnification as a single-contoured membrane. The cytoplasm of the animal consists of granular material in much larger proportion than in most terricolous forms, and accordingly presents an appearance closely simulating that of the plasmodia of various myxomycetes often present in the same cultures. In its rather dark and almost opaque cytoplasmic matrix the single nucleus is not easily to be found (PLATE 7, A, d), yet at opportune moments it can be discerned as a prolate elliptical body composed of a central hyaline part surrounded by a slightly darker external layer (PLATE 7, A, n). While the animal thus conforms to Penard's broad concept of *A. terricola* it has an appearance much different from the appearance of the habitual host of either *Endocochlus asteroides* or *B. helicoides*; and would seem, moreover, to be as completely immune from being parasitized by these fungi as their habitual hosts are immune from capture by the fungus preying upon it. In view of these differences, and of what would seem to be a somewhat indiscriminate

application of Greeff's binomial, requirements for more specific reference suggest again the use provisionally of an appended numeral in the designation *A. terricola* III.

The fungus destructive to this *Amoeba* is represented in its vegetative stage by a non-septate mycelium branching at moderate intervals both within and on the surface of the substratum, the branches being directed usually at wide angles to their parent filaments in a seemingly ill-defined haphazard manner (PLATE 7, A). Here and there the hyphae show noticeable though usually not pronounced variations in diameter. The commonplace morphology of the diffuse mycelium together with the absence of visible adhesive material is not at all suggestive of a predacious habit. When, however, an animal in the course of its customary wanderings makes contact with a mycelial filament, the contact proves unexpectedly persistent and tends to increase in extent, so that a certain measure of inwrapment often results, especially in cases where additional filaments nearby are also engaged (PLATE 7, A, a). From its applied surface the mycelium buds forth at intervals delicate processes that perforate the pellicle, penetrate a short distance into the body of the animal to give rise there individually to several swollen lobules in botryoid arrangement (PLATE 7, A, a-d). Though these endozoic processes are of rather small dimensions and ordinarily do not exceed a half-dozen in number, their intrusion is soon followed by obvious degenerative changes in the animal's cytoplasm. The *Amoeba* here concerned, unlike the forms parasitized by *Endocochlus asteroides* and *Bdellospora helicoides*, shows little endurance to internal attack, usually succumbing before the cellular contents have become greatly reduced (PLATE 7, A, b, c).

If the general course of the predacious relationship can be followed without any difficulty, the precise manner of capture is a matter of inference rather than of direct observation. The stalked processes with their botryoid terminations present, it is true, much the appearance of grappling organs, but whether they actually function as such seems somewhat unlikely, as the semi-liquid or perhaps softly gelatinous consistency of the protoplasm in which they are immersed can hardly be assumed to furnish anchorage firm enough to resist the ordinary locomotor pull of the animal.

Moreover the very production of the processes must have as an antecedent intimate contact for a more or less protracted period between parent hypha and pellicle. Since this contact is maintained in spite of the animal's restricted but appreciably lively movements in all directions, it may be presumed that adhesion is operative here even though visible deposits of sticky material cannot be distinguished. In mode of application a close similarity to the conidium of *Bdellospora helicoides*, which likewise adheres to its host without an adhesive substance being visible, thus becomes apparent. The similarity is extended in an unmistakable correspondence between the endozoic parts growing from the adhering structures, in accordance with which the delicate stalked processes are interpretable as haustoria rather than as capturing organs.

In whatever the apparatus of capture may consist, as to its efficacy at least there can be no question. When a soft agar medium has been used in making a culture, the *Amoebae* move about freely in or through as well as on the substratum, and are then caught largely here and there on the submerged mycelial hyphae without becoming concentrated anywhere so as to cause much of a display. With the employment of a harder agar medium, however, the animals are constrained to live, feed and move about exclusively on the surface of the culture; and naturally it is then only on the surface that they are captured. As a result scores and even hundreds of *Amoebae* in various stages of decline or disintegration then often accumulate on the restricted areas occupied by separate mycelial tracts of the fungus, becoming readily visible to the naked eye as a superficial deposit of greyish stippled aspect. At later stages the deposit usually is obscured somewhat under a whitish pulverulent efflorescence, which upon microscopic examination is revealed as consisting of a profuse tangle of conidial chains.

In addition to their origin in catenulate arrangement the conidia of the fungus show resemblances to those of *Cochlonema verrucosum* and *Bdellospora helicoides* in their generally fusiform shape as well as in the sculpturing of their enveloping membranes (PLATE 7, A, f, g; B, a-c). The spore chains are borne on usually rather short, erect, distally attenuated branches that arise without much

regularity from the superficial hyphae. Because the chains are usually long—lengths of from .5 mm. to 1 mm. being not infrequent—relative to their width especially at the constricted connections, they usually droop until the distal portions come in contact with the substratum. On maturation they are broken up into the separate conidia when disturbed even slightly as by the jostling of a passing nematode.

Sexual reproduction of the fungus takes place abundantly in the substratum underlying the conidial chains. Two branches, one often more or less contorted (PLATE 7, b, d, e; c-g), the other more nearly straightforward in course, arising from separate elements, whether mycelial filaments (PLATE 7, b, d, e; c-h) or germinating conidia (PLATE 7, n, o), encounter one another, fusion takes place, and at the junction a spherical body makes its appearance. This body increases steadily in size, being supplied with protoplasm from both branches, with each of which it communicates directly, never indirectly through a single common prolongation as in the sexual apparatus of the parasites previously described herein. During the earlier stages the branches remain continuous, but later, usually when the globular body is well along in development, a cross-wall appears in one or both of them at some distance from the body. On attaining definitive size the spherical body is delimited from each of the branches by an approximately tangential septum (PLATE 7, g, h). Within the smooth zygosporangium thus formed is developed the zygospore proper, a distinctly yellowish structure which at complete maturity reveals a handsomely sculptured, bullate wall, a parietal layer of uniformly coarsely granular material and a central reserve globule (PLATE 7, i-m). The zygosporangial membrane generally collapses somewhat between the bullate protuberances, though remaining recognizable usually as a separate envelope.

The predacious habit of the fungus entails such a marked difference in morphology of vegetative thallus from the genera *Cochlomena* and *Bdellospora* that the proposal of an additional genus, under a name constructed from two words meaning "animal" and "anything that fixes or fastens" respectively, appears justified.

**Zoopage gen. nov.**

Mycelium effusum; hyphis ad pelliculam animalium adhaerantibus, ramulis hanc perforantibus, haustoria intus evolventibus. Conidia aeria, hyalina, fusoidea vel elongata, in catenulas saepe longas plus minusve erectas digesta. Zygosporangia globosa, intra materiam ambientem vel subjacentem ex copulatione hypharum similium orta.

Mycelium effuse, the hyphae adhering to the pellicle or integument of an animal, perforating it and producing a haustorium inside. Conidia aerial, hyaline, fusoid to elongated, arising in more or less erect chains from aerial branches. Zygosporangia globose, produced in the substratum at the junction of two conjugating hyphae.

**Zoopage phanera sp. nov.**

Mycelium ramosum; hyphis hyalinis, 1.2-2.7  $\mu$  crassis; haustoriis pedicellatis, pedicello .5-1  $\mu$  crasso, 3-6  $\mu$  longo, 3-7 lobulos turgidos saepe 2-3  $\mu$  longos et crassos ferente. Conidia minute verrucosa, elongato-fusoidea, 25-60  $\mu$ , saepius 35-45  $\mu$  longa, 2.2-2.8  $\mu$  crassa, ex apice hypharum saepius brevium oriunda, in quaque catenula quina usque vicena quina. Zygosporangia 9-12  $\mu$  diam., primum levia, maturitate nonnihil collabentia. Zygospores flavidae, 6.5-10  $\mu$  diam., loculo 5-7  $\mu$  diam., membrana .6-1.5  $\mu$  crassa 15-30 verrucis ornata.

Hab. in terra, *Amoebam terricolam* (in senso lato) capiens et consumens, Washington, D. C.

Mycelium branched; hyphae 1.2 to 2.7  $\mu$  wide; haustoria pedicellate, the pedicels, mostly .5 to 1  $\mu$  in width and 3 to 6  $\mu$  in length, bearing apically in botryoid arrangement from 3 to 7 lobulations measuring 1.5 to 6  $\mu$ , mostly 2 to 3  $\mu$ , in length and in thickness. Conidia elongated fusiform, minutely verrucose, measuring 25 to 60  $\mu$ , mostly 35 to 45  $\mu$  (average 40  $\mu$ ) in length and 2.2 to 2.8  $\mu$  (average 2.4  $\mu$ ) in width, produced in chains of 5 to 25 on distally attenuated, mostly short branches. Zygosporangium 9 to 12  $\mu$  in diameter, at first smooth, at maturity collapsing somewhat about the sculptured zygospore. Zygospore yellowish, 6.5 to 10  $\mu$  in diameter, with a locule 5 to 7  $\mu$  in diameter and a wall .6 to 1.5  $\mu$  thick; the wall provided with 15 to 30 verrucose protuberances, of which 6 to 8 are visible in profile on its outer sigillate contour.

Capturing and destroying *Amoeba terricola* (in the broad sense more particularly of Penard), Washington, D. C.

**TAXONOMIC CONSIDERATIONS**

The similarity of the involved zygospores of *Bdellospora helicoides*, even in the absence of bladder-like appendages, to the

spirally intertwined sexual branches of *Syncephalis nodosa* directs attention to the possibility of a homological relationship between the conidial chains produced in three of the newly erected genera with the rows of asexual spores characteristic of *Syncephalis*, *Piptocephalis* and *Syncephalastrum*. An important difference is, of course, at once apparent in the absence here of anything at all corresponding to the simple or branching, usually rather stout, erect hyphae whose remarkably varied capitulate development must have facilitated the plausible interpretation of the ultimate cylindrical elements borne on them as constituting either linear sporangia or sporangial ramiſcules, wherein the spores though formed in a row are nevertheless formed endogenously much as in species of *Mucor* or *Rhizopus*. It might be argued with some cogency, however, that endogenous formation of asexual spores could conceivably occur in the Zygomycetes unassociated with the easily recognizable correspondencies of external morphology through which homologies with the very familiar spherical type of sporangium have been brought into relief; that, in fine, an element approximately homologous to a linear sporangium or sporangial ramiſcule might be formed, without the intervention of a conspicuously differentiated sporangiferous hypha, directly on a commonplace filament. The proof of endogenous spore production here would necessarily depend more nearly exclusively on optical evidence, like that adduced by Thaxter (17) in the case of *Syncephalastrum racemosum* Cohn, showing physical separateness of the spore wall from a sporangial wall or ramiſcule wall enveloping it.

No such conclusive evidence bearing on sporulation in the four catenulate fungi described herein has come to light, though a few morphological details permit of possible alternative interpretations favorable to endogeny. Thus the development of spore chains from originally continuous filaments with regularly spaced constrictions might be taken to imply parallelism with the type of spore formation illustrated by Thaxter in several figures (17, pl. 1, figs. 9-11) of his *Syncephalis Wynneae*; or, on the other hand, it might be construed as representing merely a modification of the type of spore formation familiar in *Oospora lactis* Fres. Again the presence of sculpturing on the conidia of all four catenulate species, when the conidia of comparable non-catenulate *Amoeba-*

destroying fungi are uniformly smooth, might or might not be taken to indicate parallelism with *Syncephalis intermedia* Van Tiegh. and *Syncephalis nodosa*, where according to Van Tieghem (18) the wrinkled exterior is attributable to the persistence of the segment of the sporangial envelope surrounding the conidium proper.

In any case, concerning the intimate relationship of these non-catenulate *Amoeba*-destroying forms with the catenulate forms there assuredly can be no serious doubt. The strong similarity in vegetative habit between *Endocochlus asteroides* and *Cochlonema verrucosum*, supported by a general parallelism in make-up of sexual apparatus, provides adequate testimony to a close affinity between these two species. Nor are the two types of conidial production exemplified in these species entirely irreconcilable with one another. The segments resulting from the insertion of the first order of septa in the conidiiferous hyphae of *Endocochlus* can without much straining be homologized with the seriate swellings in the young spore chains of *Cochlonema*; the lateral conidia proliferated from them then becoming interpretable as the immediate exogenous products of structures equivalent to catenated conidia. The sessile conidia of three of the delicate Phycomycetes capturing rather small *Amoebae*, which were figured in my earlier note (9, figs. 2, 4, 5), appear certainly morphologically equivalent to the conidia of *Endocochlus*, even though they are produced from prostrate, predacious and hence primarily vegetative hyphae rather than from primarily reproductive aerial filaments. In the two (9, figs. 4, E; 5, E, F) of the three forms for which sexual reproduction is known, this type of reproduction is closely similar to that found in *Zoopage*, the small originally smooth zygosporangium communicating separately with each of the outwardly almost undifferentiated zygomorphic branches; and its wall later collapsing slightly over a sculptured zygospore. Precisely the same make-up of sexual apparatus is represented also in a fourth delicate predacious form whose small conidia are produced successively on a delicate erect sporophore (9, fig. 3). A simplification of this multiple proliferation of conidia appears in an *Amoeba*-capturing fungus yet to be described, which gives rise to an elongated obovoid conidium, measuring on an average about 15  $\mu$  in length

by  $6.4\ \mu$  in diameter, at the tip of an erect conidiophore usually about  $200\ \mu$  long and  $.8\ \mu$  wide. This form is of especial importance as it reveals such thoroughgoing parallelism with the much larger Phycomycete capturing nematodes by adhesion, which was figured in a brief summary (8, fig. 8), that a close natural relationship is sufficiently obvious.

Through the ramification of overlapping resemblances, therefore, the five fungi newly described are shown to be related rather intimately with forms differing greatly from them and from one another in the arrangement and dimensions of their conidial apparatus. It may be presumed that fungi of similar morphology have been encountered by investigators from time to time; yet, aside from some descriptions of filamentous growths associated especially with protozoans, hardly any mycological writings can be referred at all plausibly to the group under consideration. The *Saprolegnia* B of Penard seems almost certainly to belong here, though the production of zoospores attributed to the fungus is little consonant with development in the Zygomycetes generally. The mycelium which Dangeard (4) described as attacking an *Amoeba* by means of a bifurcating haustorium, and to which in the absence of asexual and sexual reproductive structures he applied the binomial *Rhizoblepharis amoebina*, might be referred to the group under discussion with more plausibility if dichotomous branching of the haustorium did not also occur, and, indeed, in much greater measure, in the *Amoeba*-capturing fungus figured earlier (9, fig. 1), whose freely septate mycelium and septate, appendaged conidium rather clearly indicate mucoidaceous affinities. Possibly the filamentous outgrowths that on being found attached rather consistently to two species of *Amoeba* by Leidy (11) were mistaken by him for normal appendages and thus made the basis for a separate genus *Ooramoeba*, may belong here, the chains of fusoid segments shown in the figures of *O. botulicauda* Leidy (11, pl. 9, figs. 13-17) being especially suggestive of relationship. While the filamentous attachments (*Amoebophilus Korotneffii* Dang.) that led Korotneff (10) likewise to propose a new genus of *Amoebae*, *Longicauda*, show somewhat less resemblance to the known members of the group in question, the possibility of relationship is yet not to be excluded; and the same situation ob-

tains apparently in regard to the different outgrowths (*Amoebophilus caudatus* Dang.) which Penard (14) figured and discussed as being fungi attached to *Amoeba nobilis* Pen. and *Amoeba vespertilio* Pen. Dangeard's figures of the fungus outgrowths on his *Pelomyxa vorax* to which he (5) applied the binomial *Amoebophilus Penardi* reveal *Leptomititus*-like constrictions through which a condition not greatly unlike that evident in immature spore chains of *Cochlonema verrucosum* and *Bdellospora helicoides* is brought about.

In view of the extensive study devoted to *Amoebae* for many decades the paucity of literary references to fungi possibly assignable to the series under consideration might seem remarkable, especially as much more definite descriptions and records of parasites belonging to such relatively difficult chytridiaceous genera as *Sphaerita* and *Nucleophaga* are available in some number. The explanation for this paucity very probably lies in the fact that protozoologists have very largely kept or cultivated in water the animals studied by them. All of the five newly described fungi as well as the various allied predacious forms are pronouncedly terrestrial in their asexual reproduction, if not also in their sexual reproduction and biological adaptations. It appears very doubtful whether the adhesion of conidium or filament in *Endocochlus asteroides*, *Bdellospora helicoides* and *Zoopage phanera* necessary for penetration can occur when the host animal is bathed in free liquid water. Even if vegetative development were provided for under aquatic conditions, the production of conidia, so important in the multiplication of these fungi, would generally be meager as evidently it can proceed only in the air. In cultures consisting of irrigated decaying plant materials, one of the predacious forms figured earlier (9, fig. 2) has often been seen to extend its mycelium, partly submerged, partly floating, some distance from the solid substratum, but its conidia even then were always formed in the air on floating filaments. Indeed, appearances suggest that in nature the special function of the curious empty appendages, whose presence on the conidia of this species as on those of several of its predacious allies constitutes a feature anomalous among the Phycomycetes, might be to give the buoyancy necessary to prevent

submergence in local accumulations of water following rains or heavy dews.

If aquatic conditions while facilitating observation are unfavorable for normal development of the fungi under discussion, terrestrial conditions attaching to natural substrata like moist soil, leaf mold, decaying plant remains and excrement of animals, favor abundant normal development but impose serious impediments to observation. The rather meager and scattered conidial apparatus is usually neither very conspicuous in the much more luxuriant growth of saprophytic forms often surrounding it, nor disposed in a manner to make easy the manipulations entailed in transfer to a microscopic preparation; and when successfully mounted shows little or nothing to distinguish it from that of commonplace Hyphomycetes. With sexual apparatus, vegetative thallus and all evidence of its curious parasitism concealed in an opaque substratum, *Endocochlus asterooides* might with good fortune be referred perhaps to the genus *Acladium*; while *Cochlonema verrucosum*, *C. dolichosporum*, *Bdellospora helicoides* and *Zoopage phanera* might find places among the unsifted species compiled in *Fusidium*. It is therefore in these Hyphomycetous genera and in *Oospora*, closely similar to *Fusidium*, that the fungi herein described and forms strictly congeneric with them most likely need to be looked for in the literature; though my own searches here have so far yielded no immediately useful information.

In this connection it may not be amiss to direct attention to the general similarity of conidial development in *Cochlonema*, *Bdellospora* and *Zoopage* to that described earlier (6) as occurring in *Actinomyces*, a genus formerly often included in *Oospora*. This similarity gains in suggestiveness from the circumstance that the vegetative mycelium characteristic of *Actinomyces* with its sparse septation largely if not exclusively consequent to protoplasmic degeneration in aging portions, or to withdrawal of protoplasm into younger ramifications, conforms rather well in fundamental design with that of the Phycomycetes. The extraordinary delicacy of the mycelium in *Actinomyces*, while very little reminiscent of the more familiar types of fungi among the Oömycetes and Zygomycetes, is, as has been mentioned, matched in the extremely slender hyphae of the minute *Amoeba*-capturing forms concerning

whose close relationship in the Phycomycetes to the three catenulate genera herein described, there can be no reasonable doubt. To be sure the conidia of none of the more minute *Amoeba*-capturing forms now known are either catenulate or of dimensions at all comparable in smallness to the filaments from which they are produced; and in the known members of the catenulate genera neither the filamentous parts nor the conidia can be regarded as especially small. Yet when, as in the present instance, an obviously natural group embodies extreme delicacy of mycelium in some members, and in others formation of catenulate conidia not greatly exceeding in diameter the hyphae on which they are borne, the possibility of its relationship to forms wherein both these features are combined deserves consideration. Until the affinities of *Actinomyces* are definitely revealed through the discovery of a convincing sexual stage—the “Vierhyphensporen” described by Lieske (13) are far from impressive when viewed in such character—the morphological resemblances just noted would seem to compare more than favorably with the similarities that have long been cited as justifying the relegation of the genus to the higher bacteria.

For the time being the disposition of the group embracing the four newly described genera and the forms obviously kindred to them presents a more immediate problem. The similarities and possible homologies suggested more particularly in a comparison between *Bdellospora helicoides* and *Syncephalis nodosa* would seem to betoken articulation with the Mucorales through the Piptoccephalidaceae. On the other hand the sturdy nematode-capturing form (8, fig. 8), in the moderate and often even meager development of its vegetative or predacious mycelium, in the habitual migration of its protoplasm from old hyphae to newly proliferated branches, in the large dimensions of its conidia, and in the repetitional production of secondary conidia from primary ones, reveals features suggestive of some sort of interdigitation with the Entomophthorales. What would seem to make for a provoking analogy with the latter order is apparent also in the semi-predacious behavior, as it were, of many species of *Empusa* in fixing their enfeebled insect hosts to the substratum through the production of adhesive rhizoids. A taxonomic position somewhere between the

Mucorales and the Entomophthorales is thus indicated for the group, which it is believed deserves recognition as a separate family, to be designated with perhaps tolerable appropriateness as the Zoopagaceae. Indeed, there is some reason to believe that further study of fungi destructive to terricolous microscopic animal life under approximately natural conditions on suitable transparent solid substrata, will, without impairing the distinctiveness of the group, bring to light a range in morphological diversity and a plenitude of species commensurate in the Phycomycetes with the taxonomic scope of a suborder or of an order rather than with that of a family.

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### EXPLANATION OF PLATES

#### PLATE 1

*A*, A well developed and active specimen of *Amoeba terricola* I, showing: *a*, penetration by an infective germ tube from an adhering conidium of *Endocochlus asteroides*; *b*, development of a globular body at tip of infective germ tube; *c*, separation of the globular body or young vegetative thallus from the evacuated germ tube, the former remaining within the animal, the latter being expelled; *d-i*, young thalli within the animal in increasingly advanced stages of development; *n*, nucleus of host animal; *v*, contractile vacuole of host;  $\times 1000$ . *B*, An animal with two vegetative thalli which have given rise to three pairs of zygomorphes and a conidiiferous hypha shown in three sections.—*a* and *b* representing corresponding points on these sections, and the dotted line indicating the point of emergence from the substratum;  $\times 500$ . *C*, An animal with a single well developed thallus that has given rise to a branching conidiiferous hypha with conidia in various stages of development, the branches being shown in sections,—*a-g* indicating corresponding points on these sections, and the dotted line the point of emergence from the substratum;  $\times 500$ .

#### PLATE 2

*A*, A specimen of *Amoeba terricola* I, shortly before succumbing to infection from two well developed thalli of *Endocochlus asteroides*. *B*, An animal soon after its death from the three or four thalli of various sizes massed together in its interior. *C*, Remains of an animal containing eight thalli of *E. asteroides* of small and moderate sizes which have become evacuated in giving rise to seven zygosporangia, *a-g*. *D*, Wrinkled pellicle of an animal enveloping and concealing the membranes of the thalli which have produced four zygosporangia, *a-d*, approaching maturity. *E*, Remains of an animal showing eight mature stellate zygosporangia, each loosely enveloped by the collapsing zygosporangial membrane, *a-g*. *F*, A pair of zygomorphic hyphae with a nearly fully grown zygosporangium produced on a short prolongation from the junction. *G*, A portion of conidiiferous hypha showing *a*, an early stage, and *b*, a later stage in the lateral proliferation of a conidium. *H*, An intermediate stage in conidial development. *I*, An advanced stage in conidial development. *J*, Mature conidia, *a-f*, showing variation in shape and size. Magnification  $\times 1000$  throughout.

#### PLATE 3

*A*, Pellicle of an unidentified *Amoeba* containing an empty conidium of *Cochlonema dolichosporum* and attached to it, the thallus, also empty, produced from it. *B*, Conidia of *Cochlonema dolichosporum*, showing variation in size and shape, arrangement in chain, and appended condition at

late maturity. *C*, Pellicle of a specimen of *Amoeba sphaeronucleolus* enveloping a single thallus of *Cochlonema verrucosum* with the conidium from which it had origin still attached, and showing the basal portions of the conidiiferous hyphae to which it gave rise. *D*, *Amoeba sphaeronucleolus* about at point of death from the parasitism of three internal thalli of *Cochlonema verrucosum*, two of which have given rise to normal hyphae, while the third, through somewhat abnormal development, has developed externally a thallus-like swollen hypha. *E*, A dying specimen of *Amoeba sphaeronucleolus*, showing in addition to its two contractile vacuoles and its nucleus, three thalli of *Cochlonema verrucosum*, two of which have each given rise to an immature conidial chain shown in sections, whereof *a*, *b* and *c* represent corresponding points. *F*, Mature conidia of *Cochlonema verrucosum*. *G*, Remains of a specimen of *Amoeba sphaeronucleolus* with two zygosporangia of *Cochlonema verrucosum*, one still growing, the other containing a nearly mature zygospore. Magnification  $\times 1000$  throughout.

#### PLATE 4

*A*, Same as Plate 3, *C*, but showing the four conidial chains, *a-d*, in their entirety,—*b* and *c* being still in course of development, *a* and *d* being approximately mature; and the points of emergence of the conidiiferous hyphae from substratum being indicated by dotted lines;  $\times 500$ . *B*, Same as Plate 3, *A*, but showing the asexual reproductive apparatus of *Cochlonema dolichosporum*, consisting of three conidial chains, *a-c*, in its entirety; points of emergence of conidiiferous hyphae into the air being indicated by dotted lines; the considerable lengths of the portions of conidiiferous filaments submerged in the substratum being due to the depth at which the host animal succumbed;  $\times 500$ .

#### PLATE 5

*A*, Conidia of *Bdellospora helicoides*, showing variation in size and shape. *B*, A specimen of *Amoeba terricola* II with nine infections from separate conidia of *Bdellospora helicoides*, the separate conidia being designated alphabetically *a-i* approximately in the order of their respective stages of development into swollen vegetative bodies; *n*, nucleus of animal; *v*, contracting vacuole. *C*, Another specimen of *Amoeba terricola* II, still alive, on which have developed six swollen spore bodies, which have given rise to four zygosporangia, *a-d*; one pair of the bodies, through branching of their respective sexual hyphae having given rise to two pairs of zygophores. *D-H*, Approximately mature zygosporangia and zygospores of *Bdellospora helicoides*, together with portions of the zygophores. *I*, Remains of a specimen of *Amoeba terricola* II and of vegetative bodies of *Bdellospora helicoides* from which have been produced eleven zygosporangia of which eight, *a-h*, contain each a normal mature zygospore, while the remaining three, *i-k*, are empty as a result of internal degeneration. Magnification  $\times 1000$  throughout.

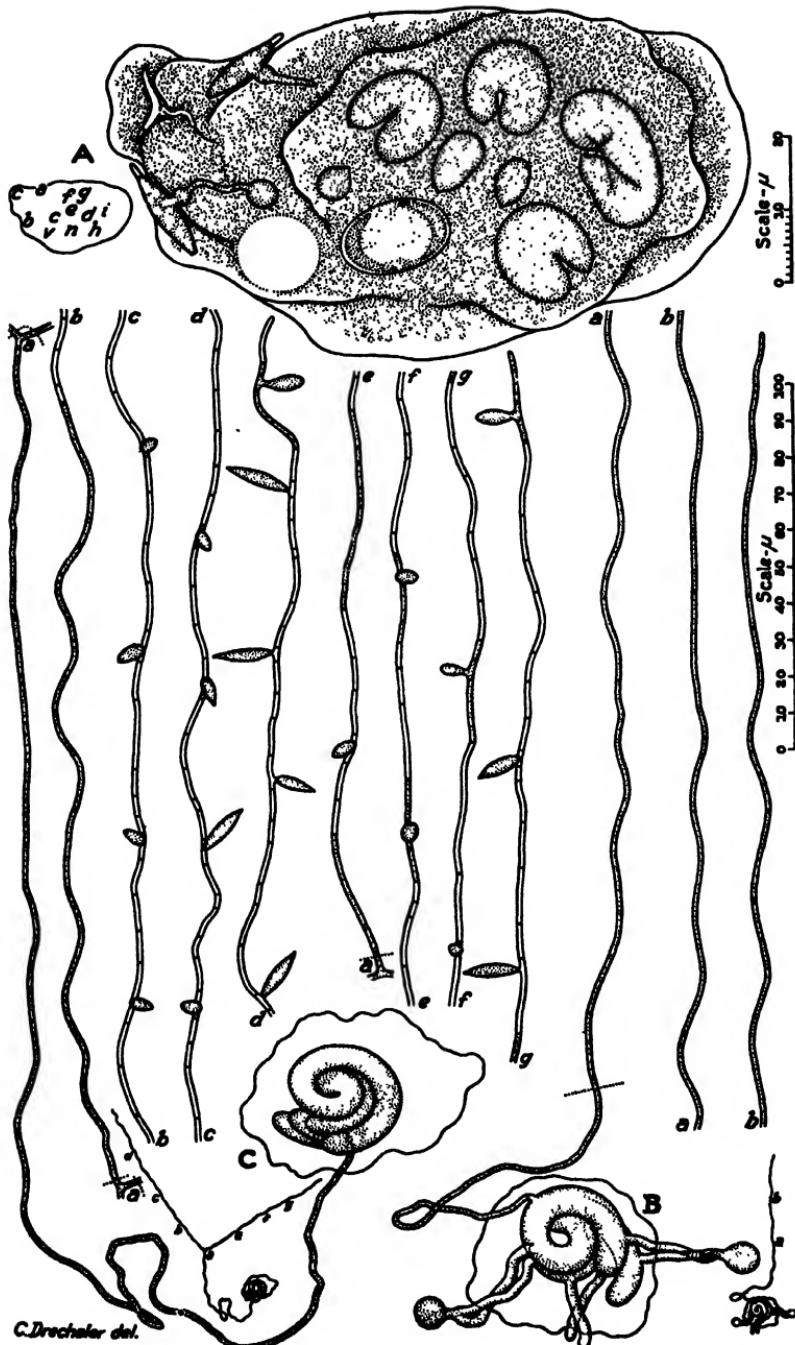
#### PLATE 6

A large specimen of *Amoeba terricola* II about to succumb to four infections from *Bdellospora helicoides*, the four swollen spore bodies having

given rise to hyphae *A*, *B*, *C* and *D*, on which have been or are being produced chains of conidia *a-d*, *a-d*, *a-b* and *a* respectively; of these chains *A*, *b* and *D*, *a* are continuous and therefore immature. The dotted lines at *A* and *C* and the lower dotted lines at *B* and *D* indicate the points of emergence of the hyphae from the substratum, the upper dotted lines at *B* and *D* the divisions between prostrate and erect portions.  $\times 500$ .

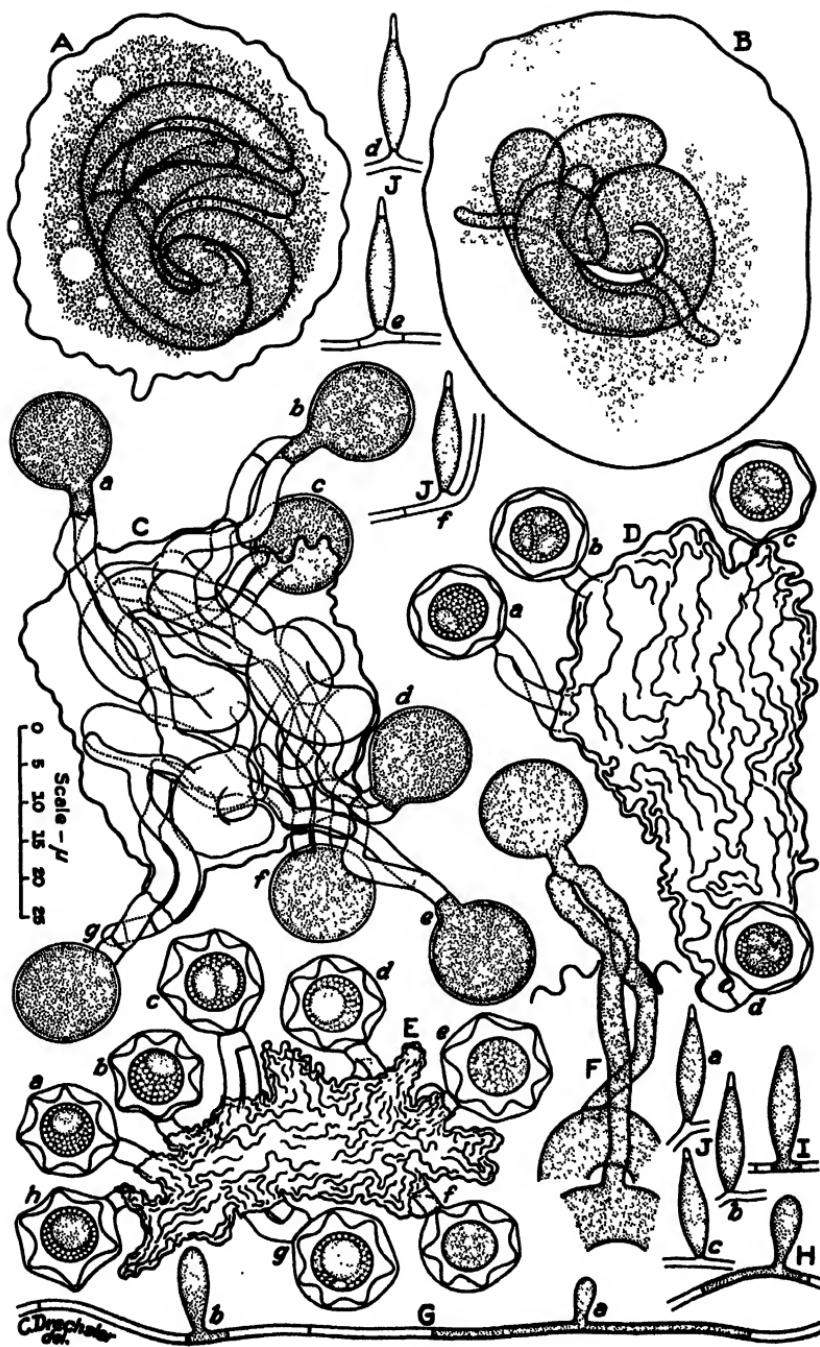
#### PLATE 7

*A*, Four specimens, *a*, *b*, *c* and *d*, of *Amoeba terricola* III captured by the branched hyphae, *e* and *h*, of *Zoopage phanera*, showing the stalked botryoid haustoria of the fungus; hyphae *f* and *g*, perhaps also adhering to animal but without having produced haustoria, bear respectively one and two erect conidiiferous branches with long chains of conidia of which from lack of space only the lowermost individuals are shown; in the animal *d* is shown its nucleus, and the same structure from a healthy specimen is shown in *n*;  $\times 500$ . *B*, Two hyphae, bearing three conidial chains *a-c* (shown only in part from lack of space) and producing on dclinious sexual branches two zygosporangia, *d* and *e*, shown at early stages of development;  $\times 500$ . *C-H*, Sexual apparatus of *Zoopage phanera* showing dclinious origin of zygophores, inconstancy of septation during earlier stages in development of fusion product, and the frequently contorted condition of one of the zygophores;  $\times 1000$ . *I-M*, Approximately mature zygospores, each within its collapsing zygosporangial membrane;  $\times 1000$ . *N, O*, Sexual apparatus, with a germ tube from a conidium functioning directly as a zygophore;  $\times 1000$ .



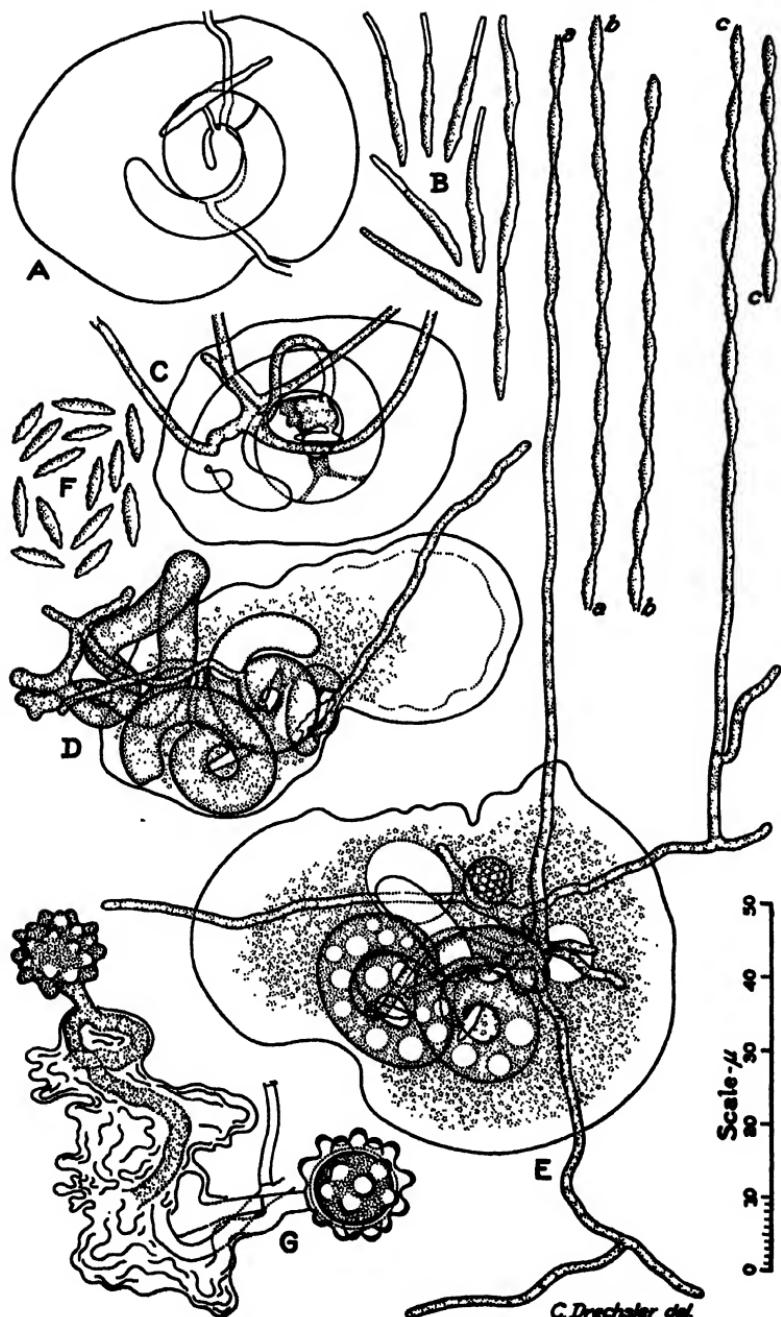
ENDOCOCHLUS ASTEROIDES





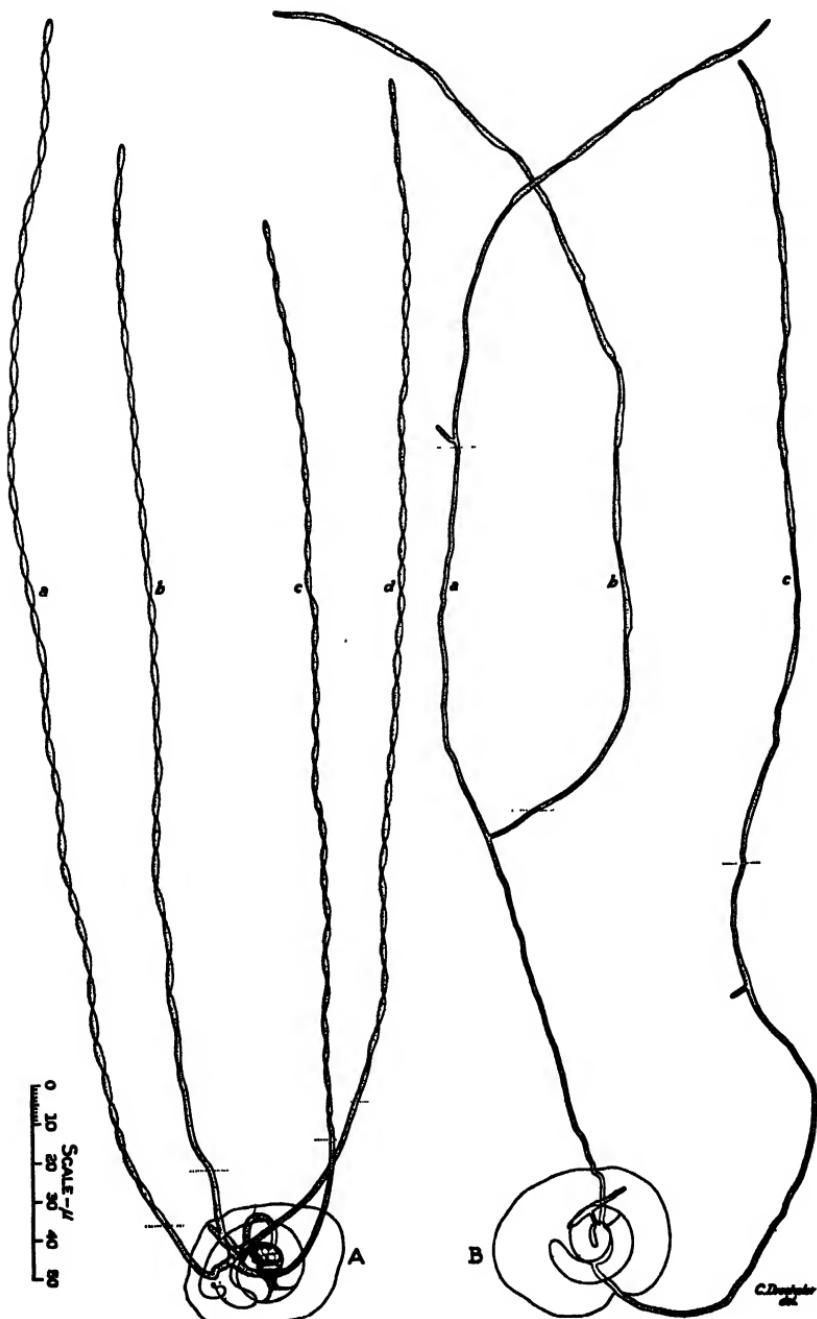
ENDOCOCHLUS ASTEROIDES





A-B. *COCHLONEMA DOLICHOSPORUM*  
C-G. *COCHLONEMA VERRUCOSUM*

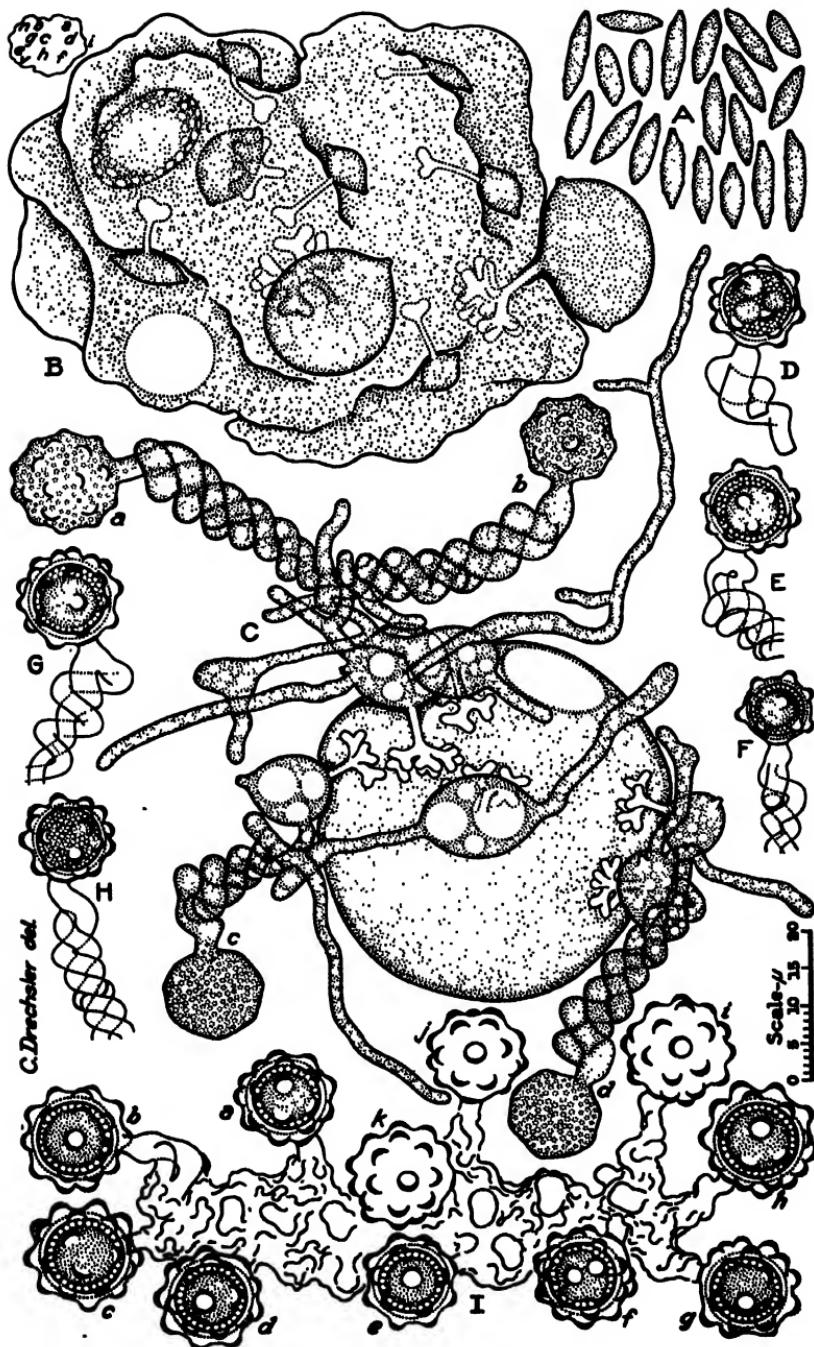




A. *COCHLONEMA VERRUCOSUM*  
B. *COCHLONEMA DOLICHOSPORUM*

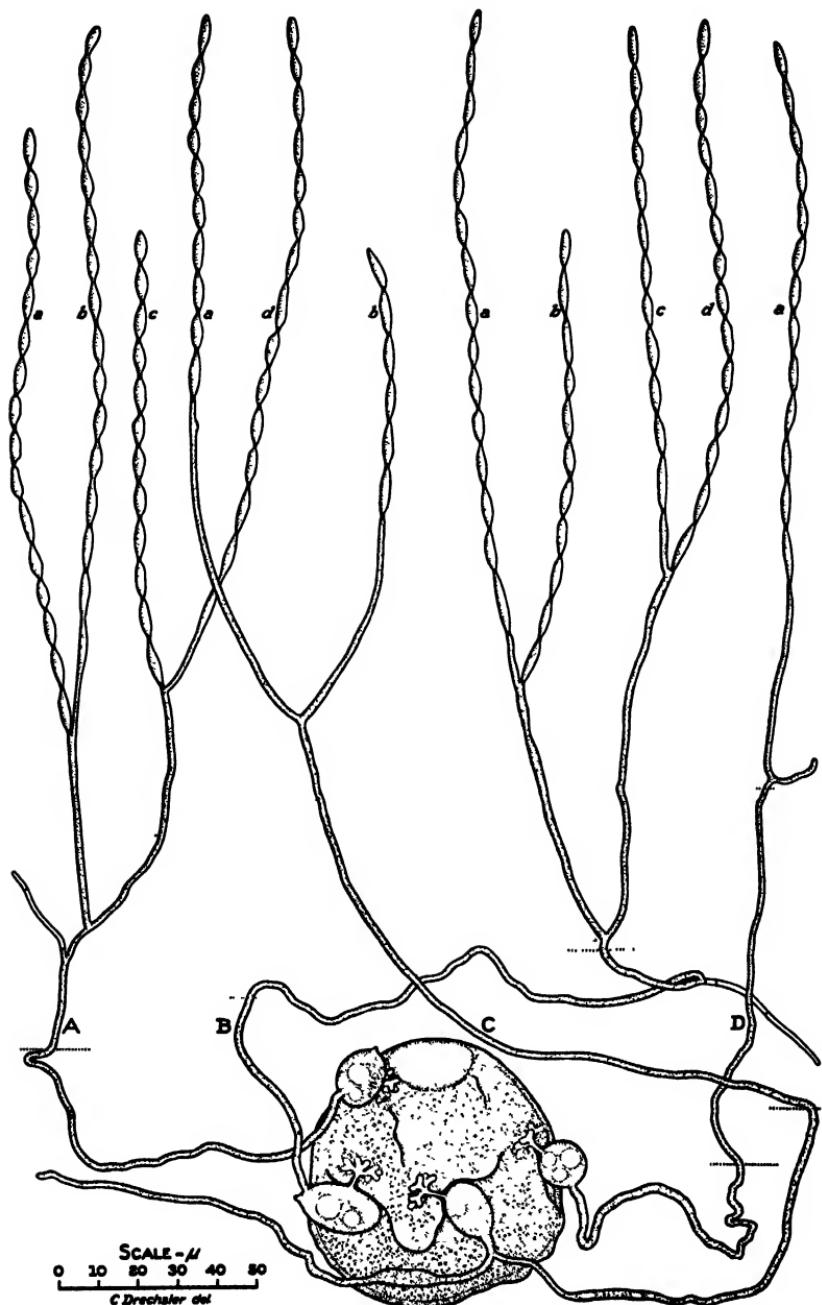
C. Dodge





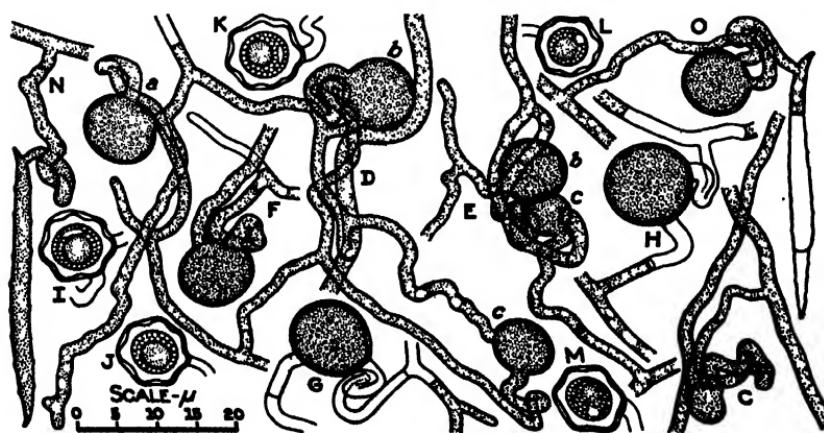
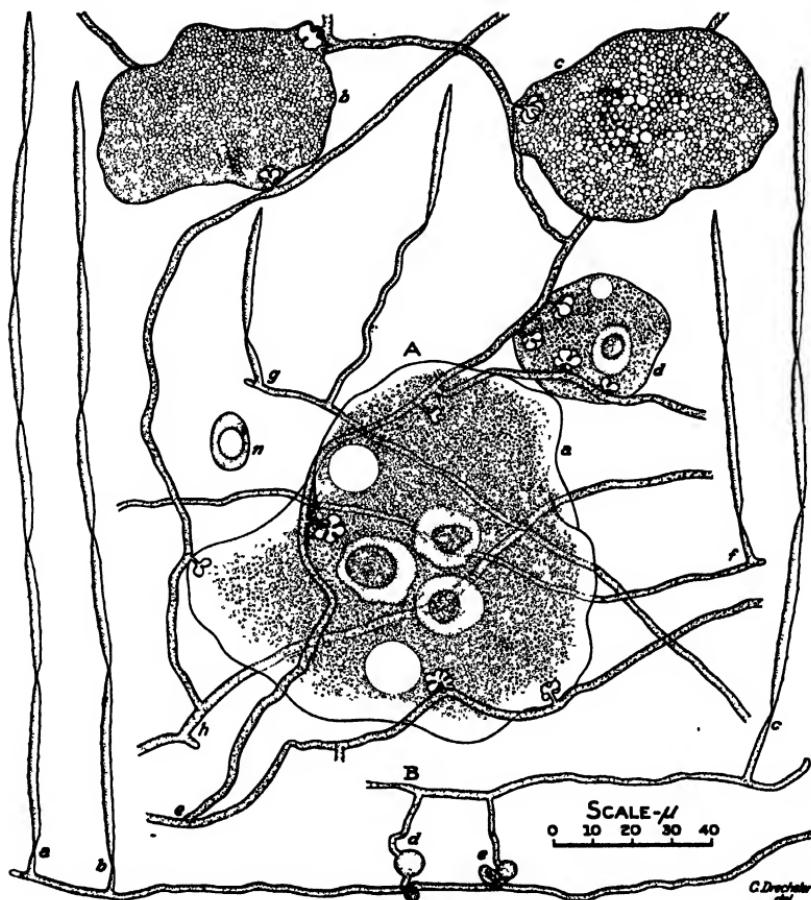
BDELLOSPORA HELICOIDES





*BDELLOSPORA HELICOIDES*







# CYTOLOGICAL STUDIES IN THE TREMELLACEAE II. EXIDIA<sup>1</sup>

Roy M. WHELDEN

(WITH PLATES 8-11)

It is most surprising in view of the fact that *Exidia* is generally recognized as the most abundant genus of Tremellaceae, that there has been so little published on its cytology. The reason for this soon becomes apparent when one undertakes such a study, for, as Neuhoff has stated, (Transl.) "For cytological treatment with the fixing solutions and stains used the species of *Exidia* are only slightly suitable since for the most part they take up dyes very poorly, so that after I began my researches with members of this genus, I had nothing to record in the first months except failure." With this statement the writer does indeed agree, for it was only after months of trials resulting in almost complete failure, that the successful methods explained fully in a previous paper (5) were developed. Even then failure to obtain satisfactory sections was not infrequent. Eventually, however, the writer obtained some three hundred collections of fruit-bodies of the various species of *Exidia* occurring in New England, i.e., *E. glandulosa* (Bull.) Fries, *E. recisa* Ditm., *E. nucleata* (Schw.) Burt, and *E. saccharina* Fries, from which, using material collected and fixed in the field, many hundreds of satisfactory sections were cut and studied. From this material, the cytological facts here presented have been drawn.

## HISTORICAL BACKGROUND

In the earlier papers one finds occasionally information other than taxonomic; for example, Sautermeister (4), in 1876, noted that there occurred on the upper surface of *Exidia recisa* Fries tubercles of filaments and paraphyses, and also that in succession on the same stroma first mature acrogenous spores (conidia) and

<sup>1</sup> Contribution No. 133 from the Laboratories of Cryptogamic Botany of Harvard University.

later "Schlauchfrucht" developed. Several years later, in 1888, Brefeld (1) studied the members of the genus and on the basis of the straight cylindrical form of the secondary spores of *Exidia saccharina* separated this as *Ulocolla* from the other species, which have sickle-shaped secondary spores.

Ten years later Juel (2), in 1898, studied various fungi, among them *Exidia truncata* Fries. While he could not find the preliminary stages, he did note the presence in the basidium of a fusion nucleus which presently divided in two reduction divisions to form four small nuclei located near the center of the basidium.

Recently Neuhoff (3), in 1924, undertook to make a comprehensive study of the various Tremellaceae, among them *Exidia*. In no case did he obtain a complete connected story of any species: in *Exidia glandulosa* and also in *E. truncata*, he elaborated on Brefeld's results, noting the uninucleate condition of the small secondary spores and also the formation of the primary mycelium with its uninucleate segments; in *E. repanda* he found little worthy of note other than the sole case of fusion between hyphae which he saw in any species of *Exidia*; in *E. saccharina* Fries he obtained more complete results, noting the presence of a transversely oriented spindle in the hypobasidium, and observing that nuclear migration from the latter through the epibasidia occurred with "no change in form," and finally that binucleated spores occur (his figure 31 of pl. 4 shows these to be two-celled).

With his paper cytological study of the species of *Exidia* seems to have lapsed. There is an imperative need for a more intensive study of the species in order that as complete a picture of the development as possible may be obtained.

#### EXIDIA

Four species of *Exidia*, all occurring rather commonly in New England, have been studied intensively in the present work. Of these species, three, *E. glandulosa* (Bull.) Fries, *E. recisa* Ditm., and *E. nucleata* (Schw.) Burt, are found very frequently on almost all broad-leaved woody plant stems and branches, while the fourth, *E. saccharina* Fries, appears to grow exclusively on the branches of *Pinus Strobus* L.

### Habit

In the youngest condition the fruit-bodies of all four species are so closely similar as to be indistinguishable, and are hemispherical, smooth-surfaced, transparent greyish bodies which in section are composed entirely of branching, frequently anastomosing hyphae, 1–1.5  $\mu$  in diameter, densely protoplasmic and with binucleate segments (PLATE 9, FIG. 1C; PLATE 10, FIG. 1, 2; PLATE 11, FIG. 6, 7). Developing fruit-bodies very quickly gain that color which is characteristic of the mature fructification, reddish-brown to black in *E. glandulosa*, clear red-brown in *E. recisa*, Auburn brown<sup>2</sup> in *E. saccharina*, and Cinnamon Buff in *E. nucleata*.

With increasing size of fruit-body come certain slight but definite changes in the hyphal structure therein. In *E. glandulosa*, hyphal branching is relatively infrequent; the hyphal segments become quite elongate and frequently multinucleate due to rapid repetition of nuclear division (PLATE 8, FIG. 1); anastomoses occur frequently, the hyphal ends enlarging slightly during the process (PLATE 8, FIG. 2). Division of the small apparently structureless nuclei shows four very evident small chromosomes which split longitudinally; while not invariably so, such nuclear divisions are frequently noted as occurring in connection with clamp formation. Such nuclear divisions almost invariably occur in the hypha at the point from which the clamp forms. As the clamp develops, one or rarely both nuclei resulting from division migrate into the base of the clamp; only one nucleus passes through the clamp, however, and enters the adjoining hyphal segment, now separated from the next by a septum (PLATE 8, FIG. 3). Much the same condition obtains in the hyphae of *E. recisa*, except that they exhibit as a rule a much more twisted condition (PLATE 9, FIG. 1, 2). *E. saccharina* shows much more frequent anastomoses (PLATE 10, FIG. 5), almost invariably associated with nuclear division (PLATE 10, FIG. 4) and with apparent migration of nuclei from one hypha to the other (PLATE 10, FIG. 6); and also shows a peculiar "prong," devoid of protoplasm, projecting stiffly out from a clamp connection (PLATE 10, FIG. 3). In *E. nucleata*, however, a quite different structure is found; for here, a lateral branch

<sup>2</sup> Capitalized color names are from Ridgway's Color Standards and Nomenclature.

develops, pushing the main filament to one side and becoming cut off, leaving the main filament quite definitely angled. A second branching may occur in similar fashion but the original segment, no matter how many times angled, remains binucleate, for a considerable length of time (PLATE 11, FIG. 7). Anastomoses between distinct branches are generally frequent (PLATE 11, FIG. 5), and are usually unique in that one of the two tips becomes much enlarged, appearing more or less to surround the other (PLATE 11, FIG. 8). Clamp connections (PLATE 11, FIG. 2) as well as the "prongs" previously mentioned also occur in this species (PLATE 11, FIG. 4), as does another structural feature, the white "kernels" of calcium oxalate, around which is found a fairly compact weft of densely protoplasmic infrequently branched hyphae.

Should the fruit-bodies of the various species of *Exidia* be subjected to periods of desiccation at this stage of development, very characteristic surface changes appear. In *Exidia glandulosa* and *E. saccharina* the fruit-bodies lose most of the 95 per cent or more of water they contain and shrink to a dry leathery patch tightly pressed to the surface of the substratum. Sections show that the hyphal tips at the surface of the fruit-body have become coarse, empty, thick-walled objects which branch frequently and are interlaced to form a tight network of hyphae spreading laterally over the entire surface (PLATE 8, FIG. 36, 37; PLATE 9, FIG. 34, 35; PLATE 10, FIG. 39). Fruit-bodies of *E. recisa* differ in remaining rigidly projecting from the substratum in much the same shape as when wet; internal structure accompanying this drying out shows the ends becoming thick-walled and devoid of protoplasm, but remaining separate and somewhat perpendicular to the surface rather than forming a thick interlacing layer (PLATE 9, FIG. 35). In *E. nucleata*, finally, relatively slight modification occurs on drying, the surface hyphae branching more frequently, becoming full of drops of "oil" and of small diameter, but not developing thick walls (PLATE 11, FIG. 34).

#### *Basidia*

The hymenium covers the exposed surface of the fruit-body in all species excepting *E. recisa* in which it is more or less confined

to the upper surface of the unilobed fruit-body. The basidia form either at the surface or, in those fruit-bodies which have developed thick-walled hyphae, beneath these. The hyphal tips which are to develop into basidia first become distinguishable through their densely protoplasmic content and somewhat swollen appearance (PLATE 8, FIG. 4, 5; PLATE 9, FIG. 3-6; PLATE 10, FIG. 7-11; PLATE 11, FIG. 9, 10). Into these slightly swollen tips move the two small nuclei, which may occupy almost every conceivable relative position in the basidium, and which have a diameter of 0.7-0.8  $\mu$  in all species except *E. nucleata*, in which it is 0.3  $\mu$ . Here they approach one the other, frequently losing their spherical shape as they do and becoming elliptical or even fusiform. These variations in shape seem not to be due to any mutual attraction. As the two primary basidial nuclei come together, rapid enlargement of the hypobasidium initial begins; this enlargement shows certain rather definite specific characters. In *E. glandulosa*, the hypobasidium becomes blunt, somewhat coarse and with uniformly dense protoplasm, which abruptly thins at the point where the basal septum will presently separate the basidium from the hypha (PLATE 8, FIG. 5). In *E. recisa*, the hypobasidium, almost from the first, becomes a fusiform body, broadest in the middle where the two nuclei are located (PLATE 9, FIG. 5). *E. saccharina* shows a hypobasidium with sides for a considerable time almost parallel, and with the apex containing a dense cap of protoplasm (PLATE 10, FIG. 8). In contrast to the other three species, the hypobasidium of *E. nucleata*, which is at the tip of a long, almost straight, slender stalk, enlarges to a subspherical body considerably smaller than that in the other three species (PLATE 11, FIG. 10).

As this enlargement begins, the two nuclei fuse to form the fusion nucleus which differs strikingly from the previously mentioned small nuclei of the mycelium. Almost from its inception, this fusion nucleus shows a very definite organization quite in contrast to the mycelial nuclei in which only the nucleolus can be distinguished clearly. In the fusion nucleus, not only is the nucleolus, comprising the two fused nucleoli, distinct, but also the chromatin material is definitely arranged into distinct linear patches (PLATE 8, FIG. 6, 8-10; PLATE 9, FIG. 7, 9; PLATE 10, FIG. 12-16; PLATE 11, FIG. 11, 13-15). This chromatin is seen more and

more clearly to be located just inside the membrane of the now rapidly enlarging nucleus; the nucleolus is also located on the periphery (PLATE 8, FIG. 8). In *E. glandulosa*, the nucleolus may very rarely become extruded to a distance from two to four microns from the nuclear surface.

As the fusion nucleus reaches its maximum size, the linear patches of chromatin, now definitely distinguishable as eight in number and not arranged in a continuous spireme, gradually contract longitudinally, while at the same time, the disintegration of the nucleolus takes place. Generally the nucleolus has entirely disappeared by the time contraction is half completed (PLATE 8, FIG. 10, 15, 16). Contraction ends with the formation of eight nearly spherical or short cylindrical chromosomes about  $0.5\ \mu$  in diameter (PLATE 8, FIG. 12; PLATE 9, FIG. 8, 10; PLATE 10, FIG. 17; PLATE 11, FIG. 12, 16). Throughout this development the nucleus remains near the middle of the hypobasidium. At the time when chromosome formation is completed, the hypobasidium has reached its maximum size, but so great is the range of dimensions of this body that little importance can be attached to a mere mention of dimensions, which in *E. glandulosa* range from  $15 \times 7$ ,  $11 \times 5.5$ ,  $20 \times 4$ ,  $13 \times 7$ ,  $9.5 \times 7\ \mu$ , etc., with an average from a hundred random measurements, of  $18 \times 6\ \mu$  (in *E. recisa* similar measurements give an average of  $12.1 \times 6.7\ \mu$ ; in *E. saccharina*,  $10.3 \times 6.1\ \mu$ , while in *E. nucleata*, where much greater uniformity exists, the dimensions are  $7 \times 5\ \mu$ ).

With chromosome formation the nuclear membrane disappears, leaving the mass of chromosomes usually compactly clumped near the center of the hypobasidium, while at the same time the transversely oriented and somewhat vague spindle appears (PLATE 8, FIG. 11; PLATE 9, FIG. 11; PLATE 10, FIG. 18; PLATE 11, FIG. 19). Definitely recognizable centrosomes were never seen during the present studies. To each of the two poles of the indefinite spindle four of the eight chromosomes migrate, thus definitely indicating as the reduction division the first to occur in the hypobasidium. Only in very rare instances is the first division equational, and in the few cases of this nature seen, all in *E. glandulosa*, there resulted a longitudinally directed spindle (PLATE 8, FIG. 13, 14).

The two daughter nuclei, fairly uniform in size for each species,

and from 1–1.5  $\mu$  in diameter, occupy positions more or less midway between the base and tip of the hypobasidium (PLATE 8, FIG. 19; PLATE 9, FIG. 14; PLATE 11, FIG. 17). Normally not until they are completely formed after division does the basal septum cut off the basidium from the hypha of which it was the tip. Greater irregularity attaches to the time of formation of the first longitudinal septum, which may develop, usually basipetally, before the basal septum is formed, especially in *E. glandulosa* and *E. saccharina*, or after, as in *E. recisa*, or with the greatest irregularity, as in *E. nucleata*. The formation of the second longitudinal septum follows directly after the second nuclear division in the hypobasidium.

This second nuclear division, almost always mitotic, except in those cases mentioned above, with four chromosomes apparent, normally occurs during or immediately after the formation of the first longitudinal septum (PLATE 8, FIG. 17, 18; PLATE 10, FIG. 19; PLATE 11, FIG. 18, 23). *E. recisa* is an exception in that both nuclear divisions frequently occur before septum formation begins (PLATE 9, FIG. 12, 13). Not infrequently the second division fails to occur, only two nuclei being formed and ultimately only two epibasidia (PLATE 8, FIG. 20; PLATE 11, FIG. 26, 27). The second division, like the first, is transverse to the long axis of the hypobasidium, with the result that the four final nuclei are all at about the same level near the middle of the hypobasidium (PLATE 10, FIG. 21, 22; PLATE 11, FIG. 25). The two divisions usually follow one another in rapid succession. In all species herein studied, the second nuclear division not infrequently occurs independently in the two nuclei, one nucleus often completing its division before that of the other begins (PLATE 9, FIG. 16, 17); indeed not infrequently only one nucleus divides, the result being a three nucleate hypobasidium, from which only three epibasidia will develop (PLATE 8, FIG. 34; PLATE 9, FIG. 18).

### *Epibasidia*

The formation of the epibasidia seems to be more or less independent of the events occurring in the hypobasidium. While the mature structures show certain fairly constant specific differences, the method of formation is uniform in all species studied. The

first indication of epibasidial development is the simultaneous appearance of two, three, or usually four small hemispherical bulges of the outer end of the hypobasidium (PLATE 8, FIG. 17, 19; PLATE 10, FIG. 21; PLATE 11, FIG. 21, 22). The rate of development of the epibasidia arising from a single basidium is practically equal and so continues until they have extended to the surface of the "jelly" or beyond. Certain differences observable in the mature epibasidia are as follows: In *E. glandulosa*, they are slender, slightly twisting objects 16 to 30  $\mu$  long, whose diameter increases somewhat from the basal 0.8–1.6  $\mu$  to a maximum of 2.5  $\mu$  when they project rigidly out above the "jelly" surface (PLATE 8, FIG. 32, 34); in *E. recisa*, the epibasidia, often arising close together at the tip of the hypobasidium, are slender, nearly straight objects which extend rigidly 10–20  $\mu$  or more above the "jelly" surface, at the same time abruptly expanding from the basal diameter of about 1  $\mu$  to approximately 2  $\mu$  (PLATE 9, FIG. 19–26); in *E. saccarina*, they are coarse, slightly tortuous objects whose length is determined solely by the amount of "jelly" present in the fruit-body, and whose diameter varies from 1.5–2  $\mu$  until they emerge from the "jelly," when it may increase to somewhat more than 3  $\mu$  (PLATE 10, FIG. 23–26; 36); in *E. nucleata*, the nearly straight, rather short, chunky epibasidia maintain a nearly constant diameter from 1–1.5  $\mu$  even when they extend slightly above the "jelly" surface (PLATE 11, FIG. 24; 26–28).

When the epibasidia have reached the surface of the "jelly," the nuclei begin migrating individually or simultaneously from their approximately central position in the hypobasidium, becoming at the same time more and more elongated, until, in *E. glandulosa*, they are slightly more than three times as long as their diameter, now about 0.5  $\mu$  (PLATE 8, FIG. 32) (*E. nucleata* is an exception in having nuclear migration start soon after the inception of the epibasidia). Without further change the nuclei migrate into the epibasidia, and rapidly progress to its outer end, where a pronounced alteration in shape occurs, namely, a decided extension toward the sterigma, at the tip of which extension the nucleolus may frequently but not invariably be observed (PLATE 8, FIG. 21, 34; PLATE 9, FIG. 22–25; PLATE 10, FIG. 29). In rare cases, the

nucleus may round up in the epibasidium, seeming to remain in this condition for some time (PLATE 10, FIG. 28).

During the migration of the nucleus into the epibasidium, there has developed from the tip of the latter the sterigma, a slender tapering object, which in *E. recisa* may reach a length of  $5\ \mu$ , but is generally much less (PLATE 8, FIG. 32; PLATE 10, FIG. 27). At the tip of the sterigma the spore initial first appears as a small spherical object which rapidly enlarges until it reaches the diameter of the mature spore, when elongation occurs (PLATE 8, FIG. 21-23; PLATE 9, FIG. 22-25; PLATE 10, FIG. 29, 30). As this elongation takes place the nucleus, now extremely attenuated, passes through the sterigma and into the spore (PLATE 8, FIG. 22-23; PLATE 9, FIG. 26-28; PLATE 10, FIG. 30-31; PLATE 11, FIG. 33). A trace of this nuclear elongation frequently remains even when the nucleus has practically reached its final location in the distal half of the spore (PLATE 8, FIG. 24).

As the nucleus enters the epibasidium, the protoplasmic content of the hypobasidium becomes very vacuolate; the protoplasm in the epibasidium becomes equally vacuolate after the passage of the nucleus, and when the latter enters the spore, practically all traces of protoplasm have disappeared in the basidium (PLATE 8, FIG. 34; PLATE 9, FIG. 30; PLATE 11, FIG. 33). As the spore elongates to its mature dimensions it too becomes more and more vacuolate (PLATE 8, FIG. 25, 26; PLATE 9, FIG. 29-31; PLATE 10, FIG. 32, 35, 37, 38; PLATE 11, FIG. 29, 30, 31).

#### *Mature Spores and Germination*

The spores are capable of remaining dormant but viable for long periods of time. Germination of the spores readily occurs at any time, either immediately after maturity or after a considerable time, if there be sufficient water present. (In one instance, spores of *E. glandulosa* were successfully germinated, nine months after they were collected, on a glass slide placed under a fruit-body.)

On germination of the spore, the nucleus, with four chromosomes present, divides at once, while simultaneously with the early stages of nuclear division there is formed either laterally or terminally one or two germ-tubes, into each of which there migrates

one of the very small daughter nuclei, the average diameter of which is 0.3–0.5  $\mu$  (PLATE 8, FIG. 27; PLATE 11, FIG. 31, 32). In the spore itself, there may be several successive nuclear divisions, after each of which septa will form to divide the spore into from two to four uninucleate segments (PLATE 8, FIG. 28–30). Each nucleus may migrate into a germ-tube, leaving an enucleated segment in the spore, or it may divide and only one of the resulting nuclei enter the tube; or several nuclei in succession may enter a single germ-tube. In any event, cross-walls will eventually form so that the densely protoplasmic germ-tube is divided into one to several usually sickle-shaped (in *E. saccharina* straight cylindrical) secondary spores, 1–2  $\mu$  in greatest diameter and 3–3.5  $\mu$  in length, into which the very small nucleus migrates (PLATE 8, FIG. 30, 31, 33). Rarely this nucleus divides there, and a cross-wall is formed to produce a two-celled secondary spore (PLATE 8, FIG. 33). All attempts to germinate these secondary spores failed.

At times certain exceptional developments are noted. Not infrequently and apparently caused by sudden wetting, following partial drying, a basidial initial starts growing into a single tube, into which each of the primary nuclei migrate without fusing. At other times coarse hyphal tips arising from the subhymenial hyphae project rigidly up through the hymenium; at times the whole exposed surface of the fruit-body is covered with these densely protoplasmic hyphal tips, which have been particularly noticed in *E. recisa* and *E. saccharina* (PLATE 9, FIG. 32, 33; PLATE 10, FIG. 34). Subsequent behavior indicates that these may be active in the formation of the "jelly" of the enlarging fruit-body.

#### DISCUSSION

The results brought out in this paper lead to the conclusion that the conflicting statements made by the few previous workers result from insufficient material. Certainly Neuhoff's emphasis on the scarcity of interhyphal fusions is not borne out by the present work; for fusions are of common occurrence in all species studied, particularly *E. glandulosa* and *E. saccharina*. In young fruit-bodies, and also in old ones in which disintegration of hyphae may be taking place, fusions are admittedly infrequent, but in actively growing fruit-bodies practically every section will show them.

As one follows the development of a young fruit-body, he sees the hyphal tips characteristic of rapidly enlarging bodies (PLATE 10, FIG. 33; PLATE 11, FIG. 1) giving place to the young basidia whose protoplasmic content is very dense. These seem never to have been described in this genus; perhaps because such basidial initials differ but little in various tremellaceous fungi. Nevertheless, except in the very earliest stage, certain quite noticeable differences are really apparent. The most conspicuous is that of shape, which ranges from bluntly club-shaped in *E. glandulosa* and *E. saccharina*, through fusiform in *E. recisa*, to long slender tips in *E. nucleata*. In addition to this, the conspicuous apical "cap" of protoplasm in *E. saccharina* is distinctive, as is also the frequent branching of the basidial hypha (PLATE 10, FIG. 15), a phenomenon rarely to be observed in the other three species studied.

These differences in shape are noticeable while the hypobasidium is still binucleate. As the two minute nuclei move together and fuse in the enlarging hypobasidium, these differences gradually diminish, and differences in size become apparent. While the relatively smaller hypobasidia of *E. nucleata* set it off sharply, the separation of the other species is only apparent when measurements of large numbers of basidia are tabulated.

As in the previously mentioned nuclear fusions, so in the present development of hypobasidium and included fusion nucleus, the few recorded facts are in the main correct. So far as the writer knows there is no detailed statement of any part of this development. As in the case of *Tremella*, here also a definite organization obtains in the fusion nucleus from the first. The chromatin material becomes organized in eight definite patches located more or less just beneath the nuclear membrane. In this region also is the rather large nucleolus which results from the fusion of the two minute nucleoli from the primary basidial nuclei. As the fusion nucleus approaches its maximum size, the nucleolus begins to disappear, and the prochromosomes to contract into the eight minute approximately spherical chromosomes. Coincident with this process, the nuclear membrane is lost, and there appears the transversely directed spindle, which as far as the present studies reveal, never stands out distinctly at any time.

The nuclear divisions of the mature fusion nucleus occur in

rapid succession, the daughter nuclei becoming definitely reorganized between them. The first of these divisions is almost invariably the reduction division. In the one observed case in which the reduction seems to come in the second division, the spindle of the first division was longitudinally directed in the hypobasidium. In general the four daughter nuclei are formed at approximately the same level, midway in the now mature hypobasidium: numerous exceptions to this may be observed, it is true, but their number is small when compared to that of normal arrangement.

In the formation of the septa which divide the hypobasidium, little occurs worthy of note. Relatively great variation in direction of septation is the only peculiarity. Equally lacking in noticeable variation is the earlier part of the formation of the epibasidia.

As these approach mature size, they are, in *E. glandulosa*, objects which definitely enlarge in diameter, and reach a length of 16–30  $\mu$ , which is much greater than that in the other three species; in these three, the epibasidia of *E. saccharina* with a diameter of 2–3  $\mu$  are relatively coarser than those of *E. nucleata*, with a diameter of 1.5  $\mu$ , and of *E. recisa*, which are generally the most slender of the three. The absolute dimensions of these epibasidia, and to a less degree, those of the hypobasidia, offer little of value in specific determination: for the length of the epibasidium is determined by the amount of "jelly" making up the bulk of the fruit-body; if the weather is wet, the "jelly" is most abundant and the epibasidia relatively longer than usual. No connection seems to exist between the divisions of the basidial nuclei and the time of formation of the epibasidia, although usually the second division takes place at about the beginning of the growth of the epibasidia.

That there is no change in shape of the nucleus as it migrates in the basidium has been stated by Neuhoff. While it is true that there is no conspicuous change, yet the writer has always noted a definite elongation apparent in the migrating nucleus; in a body so minute this may easily escape notice. However, as the nucleus approaches the tip of the epibasidium, and before it enters the sterigma, this elongation becomes very pronounced. While the minute size of the nucleus renders very difficult accurate observa-

tion of this point, the position of the nucleolus seems in no way to be determined by nuclear migration.

When the nucleus reaches the tip of the epibasidium, the spore borne thereon is usually well developed. The process of development leads first to the formation of a somewhat spherical object whose diameter is that of the mature spore; subsequently this spherical body elongates to form the typical bent cylindrical spore characteristic of all species of *Exidia*. The size of the spores offers very evident specific ranges more definitely than do any structures previously mentioned. Only infrequently do spores depart conspicuously in size from the average; *i.e.*, very small spores, obviously abnormal (PLATE 8, FIG. 35), may be formed during the later stages of desiccation.

The behavior of the mature spore on germination is quite unlike that noted in *Tremella*. In the species of *Exidia* studied, nuclear division always accompanies germination; following which, the spore becomes divided by the number of septations. It is interesting to note that these nuclear divisions frequently occur in the germ-tubes which develop singly from each of the segments of the spore. The minute size of the nuclei has made it impossible to work out the details of the process of division here. Secondary spores, sickle-shaped save in *E. saccharina*, where they are straight cylindrical, are now developed from the germ-tube. Into each of these a nucleus migrates to become the nucleus of the spore; not infrequently this nucleus, in those cases where the secondary spore becomes two-celled, then divides again. This is wholly in agreement with the facts recorded by Neuhoff as occurring in *E. glandulosa*. Further the germination of the secondary spores was not followed, since they failed to germinate under any conditions.

In addition to the differences in spore shape which separate species of *Exidia* from *Tremella*, and in the method of germination just noted, there is another very noticeable difference which appears when the fruit-body begins to dry. This is the formation in species of *Exidia* of the layer of thick-walled interlacing hyphae which form over the fruit-body. Lacking these to a considerable degree, *E. nucleata* is less sharply set off from *Tremella* than are the other species here studied.

## SUMMARY

The cytology of the four species here studied, *Exidia glandulosa* (Bull.) Fries, *E. recisa* Ditm., *E. saccharina* Fries, and *E. nucleata* (Schw.) Burt, is remarkably uniform in all particulars.

Between the usually binucleate segments of the hyphae fusions frequently occur. Clamp connections are abundant, also.

Over the upper surface of the fruit-body the young hypobasidia, each binucleate, develop. In these, nuclei unite to form the fusion nucleus. As this enlarges, very definite organization is visible, the chromatin being clearly aggregated in eight linear patches, the prochromosomes, which contract to form the eight small chromosomes.

The first nuclear division in the basidium is the reduction division, which is followed by a second homotypic division. Subsequent to this division, the minute nuclei migrate through the epi-basidia into the spore. Contrary to Neuhoff's report, the nucleus becomes definitely elongated during migration.

Nuclear division accompanies spore germination in *Exidia*. This is in contrast to the situation in *Tremella*, where no nuclear division occurs in the usual method of spore germination.

The formation of a layer of thick-walled interlacing hyphae over the surface of the drying fruit-body is characteristic of species of *Exidia*. This feature does not occur in species of *Tremella*.

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5. Whelden, R. M. Cytological studies in the Tremellaceae. I. *Tremella*. Mycologia 26: 415-435, pl. 47-49 and text figures. 1934.

## DESCRIPTION OF PLATES

## PLATE 8, EXIDIA GLANDULOSA (BULL.) FRIES

In this and all other plates, all figures have been drawn with the aid of a camera lucida, at a magnification of  $3800\times$ , and subsequently reduced to about 0.3, i.e., to a magnification of about  $1150\times$ . In addition, an absolute scale of dimension is included in all plates.

Fig. 1, hyphal segments in which the nuclei are dividing conjugately, each nucleus showing 4 chromosomes; 2, anastomosis of hyphal tips (a) appressed, slightly swollen tips; (b), clamp-like development of one tip around end of other; 3, clamp formation showing (a) nucleus dividing before entering the clamp, (b) uncommon condition in which one of the daughter nuclei migrated through the clamp before a cross-wall has formed, (c) daughter nucleus entering the clamp which passes around cross-wall of hypha, and (d) final condition; 4, formation of basidia showing various positions of the two primary basidial nuclei which in b and c are of the frequent fusiform shape; 5, 7, hypobasidium showing formation of a lateral branch; 6, 8, 9, the enlargement of the fusion nucleus with increasingly distinct prochromosomes; 10, 16, fusion nuclei from which the nucleolus has disappeared and with prochromosomes definitely formed; 11, the nucleus shows eight small chromosomes; 12, the eight prochromosomes have contracted until the chromosomes are distinct; 13, first division with its axis parallel to the longitudinal axis; 14, the second division following fig. 13; 15, prochromosomes contracting and nucleolus disintegrating; 17, the nuclei in the second division, two epibasidia forming apically, and the earliest indication of longitudinal septum; 18, a slightly later stage of the division of the nuclei and the formation of the septum; 19, the two epibasidial initials appearing; 20, an early stage in epibasidial formation; 21, an epibasidium in which the elongated nucleus is approaching the slender sterigma at the tip of which the spore is forming; 22, tip showing the entrance of the nucleus into the spore; 23, tip bearing nearly mature spore; nucleus still connected with the sterigma by a slender strand; 24, the same, with the nucleus still showing a slender strand extending towards the sterigma; 25, nearly mature spore at tip of slender sterigma; 26, mature spore showing the nucleus in early prophase; 27, mature spore with lateral germ-tube and nucleus in metaphase; 28, 29, 30, 33, spores showing septate condition subsequent to nuclear division and stages in the development of the small falcate conidia; 31, falcate conidium showing the single minute nucleus; 32, vacuolate hypobasidium showing the nuclei passing from the mature epibasidia into the maturing spores; 35, abnormally small spore formed on a drying fruit-body; 36, apical portion of irregularly branched thick-walled hypha forming over surface of drying fruit-body; 37, stages in the development of these thick-walled hyphae showing nuclear disintegration and increasing vacuolation of protoplasm.

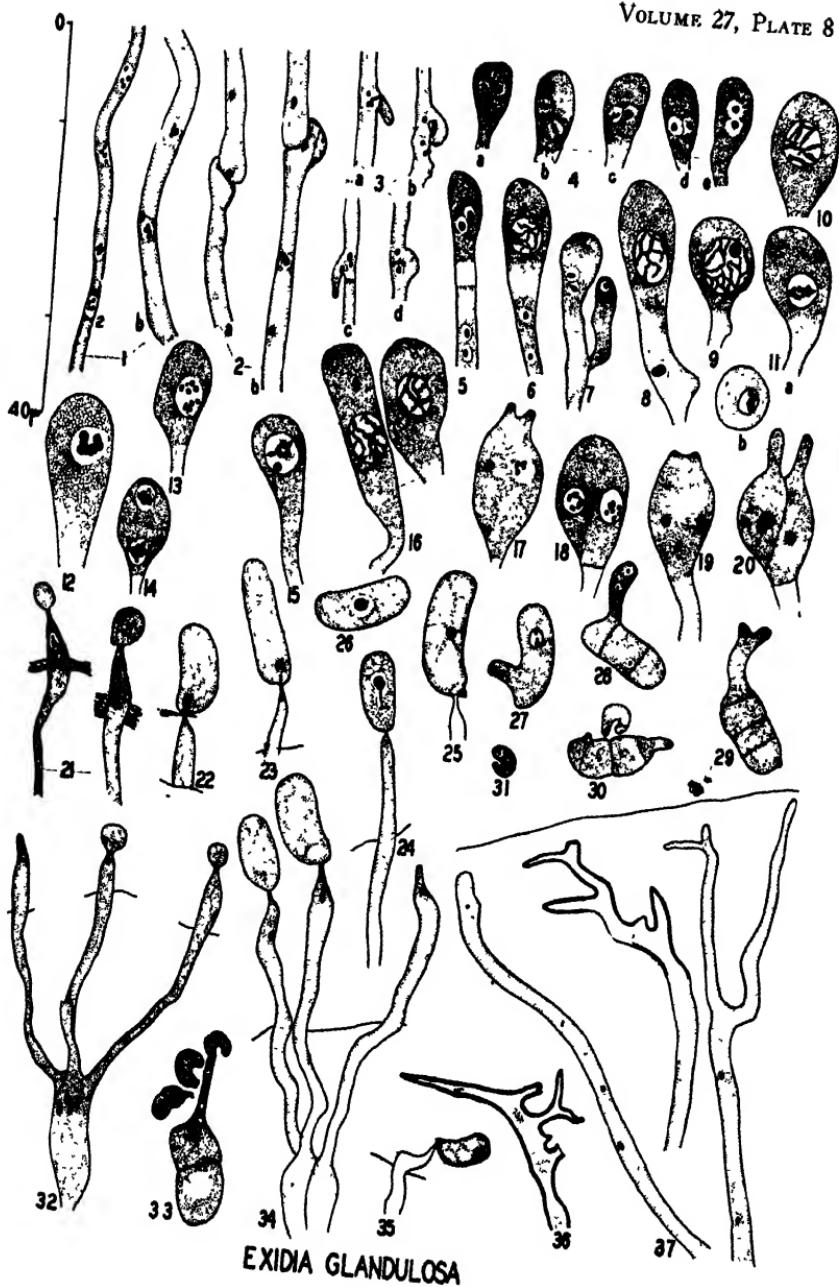
## PLATE 9, EXIDIA RECISA DITM.

Fig. 1, a, somewhat enlarged fusing apices of hyphae; b, nucleus dividing in clamp; c, "loop" clamp at end of binucleate segment of hypha; 2, clamp

forming and receiving a migrating nucleus; 3, 4, 5, fusiform hypobasidia showing two nuclei in each; 6, hypobasidium showing the nuclei just fusing; 7, an enlarging fusion nucleus and the basal septum forming; 8, eight chromosomes in the fusion nucleus, in which the prominent nucleolus is still evident; 9, the mature fusion nucleus with evident nucleolus and eight elongate prochromosomes; 10, eight small chromosomes clumped together; 11, a diagonally oriented spindle; 12, two daughter nuclei dividing simultaneously; 13, two daughter nuclei dividing consecutively; 14, two daughter nuclei; 15, abnormal hypal tips occurring among the basidia; 16, 17, small hypobasidia showing the formation of the first longitudinal septum; 18, hypobasidium showing three epibasidia originating close together; 19, four epibasidia arising apically; 20, the beginning of nuclear migration toward the epibasidia; 21, mature epibasidium showing the elongate small nucleus migrating the apex; 22, epibasidium showing the elongate nucleus approaching the tip on which a nearly mature spore has formed; 23, 24, 25, epibasidia showing successive stages in the development of the spore; 26, epibasidium tip bearing the nearly mature spore into which the nucleus is just migrating; 27, mature spore containing the pear-shaped nucleus migrating toward the center; 28, mature spore into which the nucleus is just passing from the sterigma; 29, 30, mature spores showing collapsed epibasidia on which they have formed; 31, mature spore showing the nucleus in early prophase; 32, 33, sterile hyphae which project rigidly above the surface of the fruit-body; 34, branching hypha which will become thick-walled when the fruit-body dries; 35, thick-walled hyphae which form over the surface of the drying fruit-body.

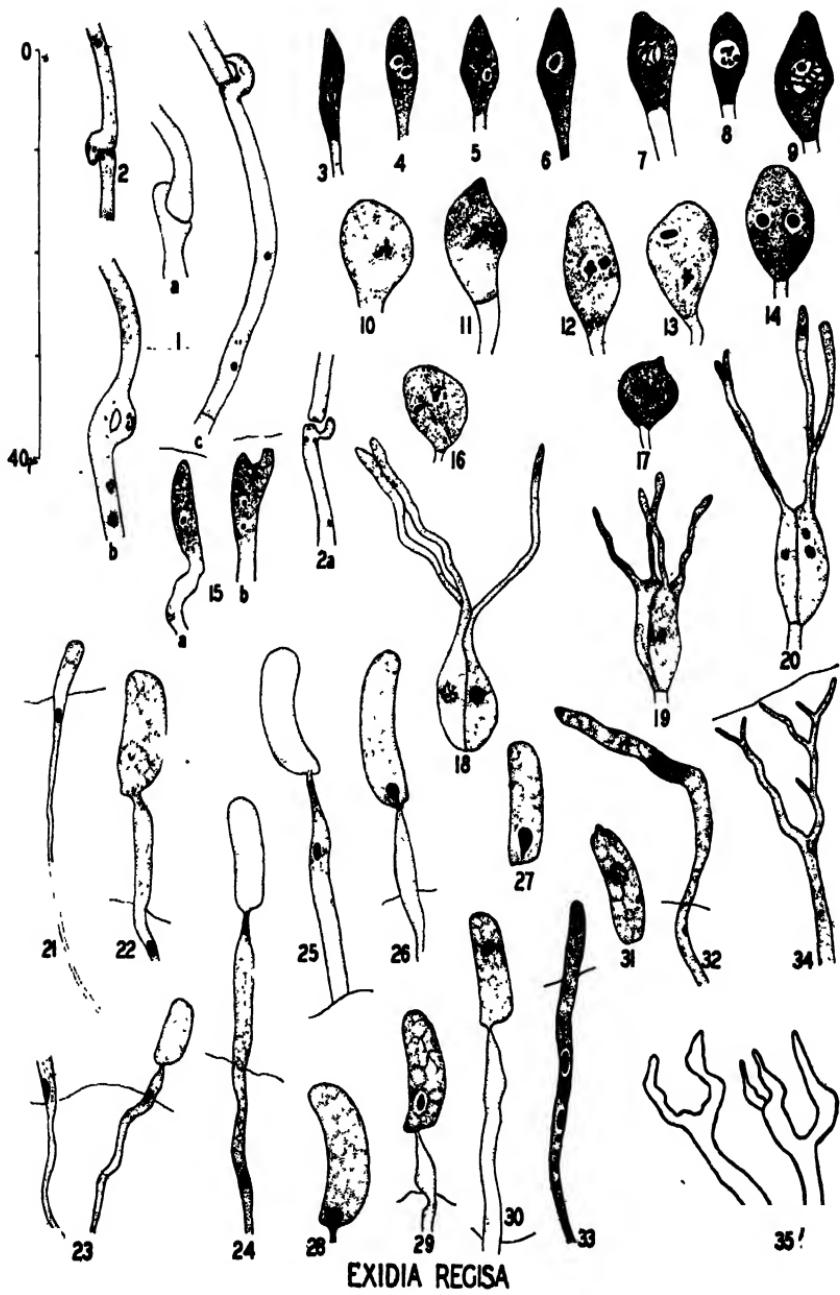
#### PLATE 10, EXIDIA SACCHARINA FRIES

Fig. 1, binucleate hyphal segment; 2, hyphal segment showing the nuclei in early prophase; 3, clamp showing "prong" projecting outward; 4, hyphal branch showing the dividing nuclei beneath; 5, anastomosing hyphal ends; 6, hyphae joined by a connecting strand at each end of which a nucleus is dividing; 7, young hypobasidium showing the two nuclei and protoplasmic "cap"; 8, a lateral branch developing from the stalk; 9, basal septum forming; 10, two nuclei just fusing; 11, forming laterally on a hypha; 12, nearly mature, showing the large fusion nucleus; 13, fusion nucleus with definite prochromosomes; 14, stage in the development of a lateral hypobasidium, later than fig. 8; 15, a complete hyphal tip showing laterally developed hypobasidia; 16, mature fusion nucleus; 17, eight chromosomes densely massed; 18, transverse spindle; 19, simultaneously dividing daughter nuclei separated by the first longitudinal septum; 20, abnormal hypobasidium showing the four daughter nuclei at about the same level and two epibasidia developing laterally; 21, normal hypobasidium showing four daughter nuclei at about the same level and the four apically forming epibasidia; 22, transverse sections of hypobasidium showing four daughter nuclei and the first longitudinal septum; 23, four epibasidia well formed prior to the second nuclear division; 24, one nucleus in division and two of the four well-developed epibasidia (the other two cut off in sectioning); 25, nuclei just entering the bases of the blunt epibasidia; 26, nuclei starting to migrate; 27, epibasidium tip tapering gradually into the sterigma; 28, nucleus below the sterigma;



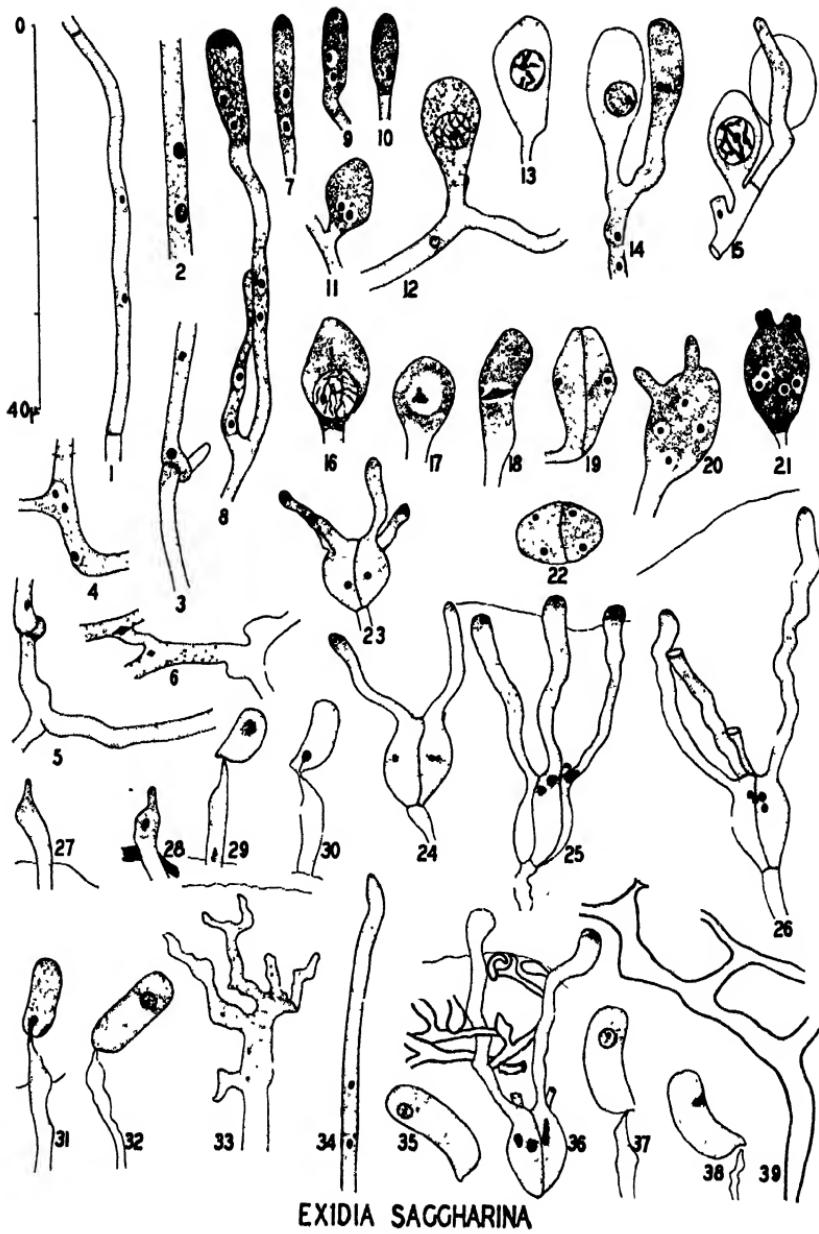
EXIDIA GLANDULOSA



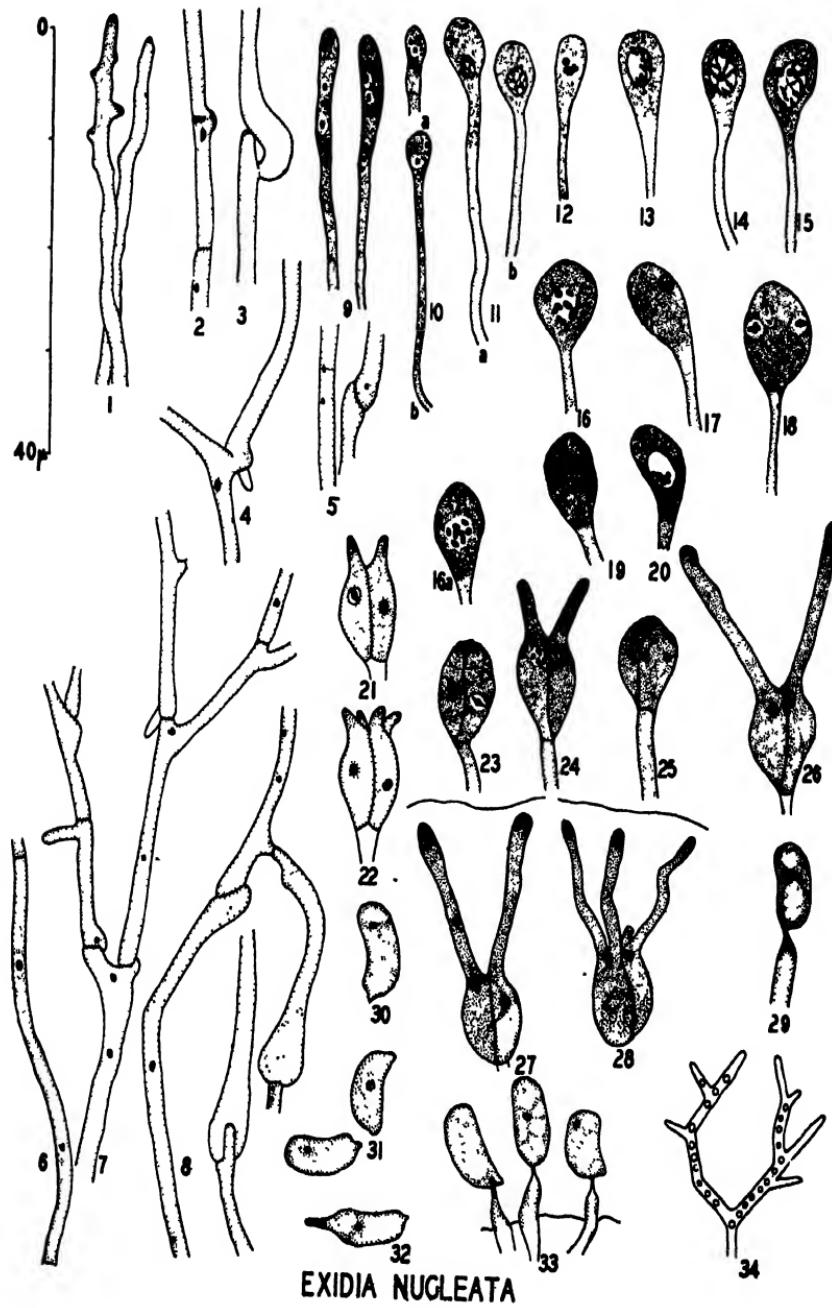


EXIDIA REGIS











29, epibasidial tip on which the partly mature spore has formed; 30, nucleus from which a slender strand extends back to the sterigma; 31, nucleus just entering the spore; 32, mature spore on the collapsed epibasidium; 33, branched sterile hypha showing numerous disintegrating nuclei; 34, unbranched sterile hypha containing two nuclei; 35, mature spore showing vacuolate protoplasm and nucleus near distal ends; 36, epibasidia pushing up through the interlacing thick-walled hyphae; 37, mature spore on collapsing epibasidial tip; 38, mature spore showing vacuolate content and dividing nucleus; 39, portion of the thick-walled hyphae which form over the surface of the drying fruit-body.

PLATE 11, *EXIDIA NUCLEATA* (SCHW.) BURT.

Fig. 1, hyphal tips in young fruit-body; 2, segments of mycelium showing a single nucleus in each; 3, apical anastomosis showing loop-like formation; 4, hyphal branch showing "prong"; 5, terminal anastomosis of hyphae; 6, normal binucleate segment of mycelium; 7, branching hypha in fruit-body showing all the nuclei dividing; 8, anastomosis showing one hyphal tip much swollen; 9, 10, young hypobasidia showing two nuclei and long slender stalk; 11, hypobasidium showing the enlarging fusion nucleus; 12, nucleus in metaphase; 13, massing of nuclear content at one side; 14, 15, mature fusion nucleus with eight prochromosomes; 16, eight distinct chromosomes; 17, two daughter nuclei near the surface; 18, daughter nuclei dividing simultaneously; 19, transverse spindle of the meiotic division; 20, chromosomes all massed at one side; 21, 23, only one daughter nuclei dividing; 22, four wide-spreading epibasidia forming apically; 24, one daughter nucleus dividing while the other is migrating toward the base of an epibasidium; 25, four small nuclei; 26, 27, two nuclei migrating to the bases of the two epibasidia; 28, four epibasidia each receiving a migrating nucleus; 29, nearly mature spore at tip of the epibasidium; 30, extremely vacuolate content; 31, nucleus dividing; 32, apical germ-tube forming; 33, three epibasidia bearing nearly mature spores, one of which is just receiving the nucleus through the sterigma; 34, branched hyphal tip full of drops of oil-like substance.

# MORPHOLOGY OF POLYTHRINCUM, CAUSING SOOTY BLOTCH OF CLOVER

FREDERICK A. WOLF<sup>1</sup>

(WITH 5 TEXT FIGURES)

In 1910, the writer first attempted to isolate the fungus that causes the sooty blotch disease of clover, but was unsuccessful. Repeated attempts have been made, without success, in subsequent years. Apparently similar failures have rewarded the efforts of all other investigators, among whom are Killian (5), Bayliss-Elliott and Stanfield (1), and Horsfall (3), who have studied this fungus. It has been necessary, because this organism is so refractory in this regard, to confine the study of its morphology and development to such phases as could be ascertained from observations made in the field and from microscopic examinations of material collected at intervals throughout the year. The findings that have resulted are not in accord, in certain features, with those of others, and are herein recorded as a contribution to a better understanding of the structure of this unusual fungus.

## SUSCEPTS

The sooty blotch fungus is widely prevalent throughout North America and Europe, and apparently is capable of attacking any of the species of *Trifolium*. No one has attempted studies to determine the suspect range, but apparently one and the same species of fungus is responsible for the disease on all species of clover. The writer has found no morphological differences in specimens of the conidial stage collected on red clover, white clover, alsike clover, and crimson clover, growing close together. Spegazzini (Mus. Nac. Buenos Aires Anal. 3: 437, 1911) ascribed

<sup>1</sup> Contribution from the Cryptogamic Laboratories of Harvard University, 119. The writer is grateful to W. H. Weston, Jr., and D. H. Linder for help in interpreting the microscopic preparations and for their suggestions and criticisms.

the name *Polythrincum Trifolii* var. *platensis* to an organism on *Trifolium platense* in Argentina. The differences that he noted do not appear, however, to warrant the retention of this varietal designation. The following enumeration of suspect species is drawn from the records of the Plant Disease Survey, which show that the fungus has been widely collected within the United States, and from the compilations of Oudemans (*Enumeratio Syst. Fung.* 1-5 vol. 1919-1924), which show that it is represented in the exsiccati of such European mycologists as Cooke, Desmazieres, Fuckel, Rabenhorst, Rouneguère, Plowright, Saccardo, and de Thümen. It has been reported to occur on the following species: *Trifolium agrarium*, *T. alpestre*, *T. arvense*, *T. elegans*, *T. filiforme*, *T. fragiferum*, *T. incarnatum*, *T. lappaceum*, *T. medium*, *T. minus*, *T. Molineri*, *T. montanum*, *T. platense*, *T. pratense*, *T. procumbens*, *T. pseudobadum*, *T. reflexum*, *T. rubens*, *T. repens*, *T. scabrum*, *T. spadiceum*, *T. stellarum*, *T. striatum*, *T. tomentosum*, and *T. Wormskioldii*.

#### HISTORY OF THE FUNGUS

The sooty blotch fungus of clover is polymorphic, possessing a conidial stage, a spermogonial stage, and a perithecial stage, and for this reason, in part, it has been variously named. In 1801, it was first described in its spermogonial stage, by Persoon (*Syn. Fung.* p. 30, 1801), as "*Sphaeria Trifolii*: atra parua, magnitudine varia, caespitulo inaequali rugoso interne subpulverulento. Habitat autumno in foliis adhuc viridibus *Trifolii* *repentis*.  $\frac{1}{2}$ -1 lin. lata. Sphaerulae farctae, intus albicantes." Neither Persoon nor the other mycologists of that period regarded spermogonia as male structures. In 1816, Schmidt and Kunze (*Deutschl. Schwämme*, no. 121) first described the conidial stage as *Polythrincum Trifolii*, as follows: "Thallus e floccis caespitosis, erectis simplicibus multiseptatis. Sporidia didyma inspersa. Hypogenum in *Trifolio pratensi* aliisque." In the following year they (Kunze and Schmidt, *Myc. Hefte* 1: 13-15. 1917) republished this description and included certain additional observations, notably that the fungus not only occurs on red clover, as shown in their specimen no. 121, but also on *Trifolium alpestre* and *T. fragiferum*, that they had often noted it in the environs of Leipzig, and that botanists had sent them specimens as *Sphaeria Trifolii* of Persoon.

Fries (Syst. Myc. 2: 435. 1923) suggested that the ascigerous stage should be a species of *Dothidea* but did not designate the clover organism as *Dothidea Trifolii* until 1849 (Summa Veg. Scand. p. 387. 1849). Even at that time he was unable to describe the ascigerous stage because, as he states, "asci hactenus frustra quaesiti" (the asci have thus far been sought in vain).

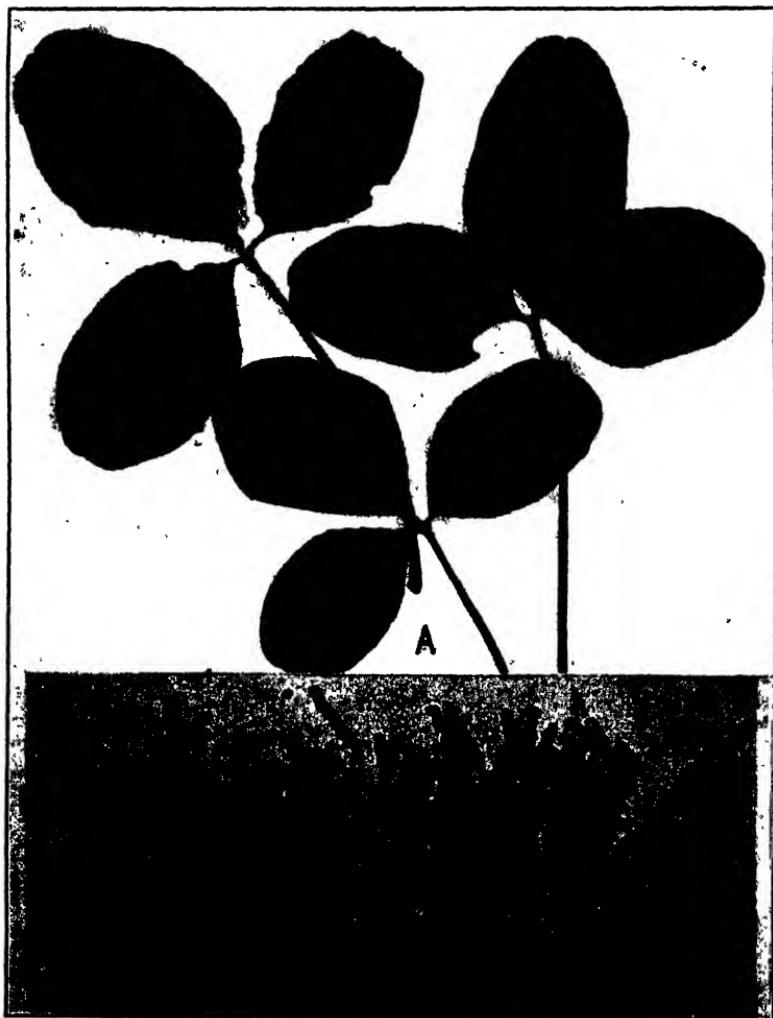


FIG. 1. A. The *Polythrincium* or conidial stage of the sooty blotch fungus of clover; B, Microphotograph of pustules of *Polythrincium* in sections cut perpendicular to the surface of the leaf.

Meanwhile Greville (Scot. Crypt. Botany 4: 216, 4 figs. 1826) employed Schmidt and Kunze's name, *Polythrincium Trifolii*, for the fungus that he noted to be of common occurrence in Scotland. The illustrations that accompany his account misinterpret its microscopic features and show that the conidiophores are articulately moniliform. They are also misinterpreted, as will be shown later in this report, by Corda (Ic. Fung. 3: 10, pl. 2, fig. 25), whose illustrations have been copied into many taxonomic works and textbooks.

In 1869, Fuckel (Symb. Myc. p. 218, 1869) transferred *Dothidea Trifolii* (Pers.) Fries to *Phyllachora Trifolii* (Pers.), with *Polythrincium Trifolii* Schm. & Kunze and *Sphaeria Trifolii* Pers. as synonyms, yet he pointed out that he had not seen the perithecial stage ("Fungus ascophorum nondum vidi"). This transfer met the approval of Saccardo (Syll. Fung. 2: 613. 1883) for the reason, as he states, that the fungus has the general habit of *Phyllachora*.

Cooke (Grevillea 13: 63. 1884-1885), in 1884, reported the presence of a specimen in the Berkeley Herbarium that possessed clavate asci bearing elliptical, continuous, hyaline ascospores,  $10-12 \times 5 \mu$ . These specimens were reexamined by Bayliss-Elliott and Stanfield (1) but they were unable to verify Cooke's observation. In 1905, Clevenger (2) recorded ascospores of similar appearance, although slightly smaller, in specimens on *Trifolium Wormskioldii*, and identified the fungus as *Phyllachora Trifolii* (Pers.) Fuckel. These specimens are the basis of Theissen and Sydow's new species, *Phyllachora umbilicata* (7, p. 510), but they stated that none of the material that they examined possessed mature ascospores.

Traverso (8), in 1903, described the spermogonial stage as a pycnidial fungus, *Placosphaeria Trifolii* (Pers.), associated with *Polythrincium Trifolii* ("socio plerumque *Polythrincio Trifolii*").

Killian (5), in France, was the first investigator to find the perithecial stage of the clover pathogen in mature condition. He found that the ascospores are bicellular, and referred the fungus to *Plowrightia Trifolii* (Pers.) because *Phyllachora* has unicellular ascospores. From investigations conducted independently and at the same time in England, Bayliss-Elliott and Stanfield (1)

found that the ascospores are 2-celled, and assigned the organism to *Dothidella Trifolii* (Pers.) since the genus *Dothidella* has priority over *Plowrightia*.

#### DEVELOPMENT OF THE PATHOGEN

In the usual course of events in the developmental cycle of the sooty blotch fungus the ascospores constitute the inoculum for the primary infections in spring. This has been shown to be the case in France from the observations of Killian (5), in England, from the observations of Bayliss-Elliott and Stanfield (1), and appears, from the writer's observations, to be the case in the vicinity of Cambridge, Mass. A collection of mature perithecia was made on the campus of Mount Holyoke College, South Hadley, Mass., on May 12, 1934, and at this time there was no evidence there of the conidial stage. In North Carolina, however, the conidial stage has been found to be produced at any time throughout the entire year, even during the winter months, but it is most abundant in early spring and late autumn. Nevertheless mature perithecia may be found in North Carolina in late April and early May on leaves that had succumbed during the previous fall.

*Conidial stage.* The conidial stage appears as punctiform, olive-brown pustules or stromata that protrude prominently from the lower surface of the leaves. At first there may be no evidence of infection on the upper surface but gradually the areas above the pustules become pale green. The pustules may remain discrete and scattered or may become closely crowded and eventually so numerous as to cover over most of the leaf surface (FIG. 1, A). Each stroma arises subepidermally as a cushion of brown pseudoparenchymatous cells. As this stromatic tissue increases certain cells at its surface elongate perpendicular to the leaf surface and form a compact fascicle of a few to approximately 30 foot-like cells upon which the conidiophores are to be borne (FIG. 1, B and FIG. 4, 1). These foot-like cells extend to the surface by rupturing the epidermis, and each bears one or more conidiophores (FIG. 4, 1 and 4, 3). The conidiophores diverge distally in a broom-like fashion (FIG. 1, B). They are non-septate and are wavy in outline.

The conidial stage derives its name from the peculiar form of

the conidiophores. This form is the result of an unusual type of sympodial branching arising from the fact that each conidiophore eventually bears and sheds one at a time a series of conidia.



FIG. 2. c, Stroma of *Cymadothea Trifolii* in vertical section showing, at the right, a spermogonium; at the left, a perithecial fundament. Numerous spermatia occur in the mucilaginous matrix that invests the trichogynes; d, Vertical section of a stroma. A group of trichogynes project from the top of the perithecial fundament, at the right; the spermogonium is shown in median section at the left.

Young conidiophores are at first straight. Conidia are borne apically, and after each one is delimited the conidiophore continues to elongate. A point lateral to the point of attachment of the conidium marks the place at which growth of the conidiophore is

to be renewed. In elongating the tip of the conidiophore is deflected because of the firmness with which the conidium is attached at its rather broad base. The last-formed conidium is therefore dislodged as the result of the lateral pressure exerted upon it by the elongating conidiophore. Circular scars on the conidiophores mark the points from which the conidia were dislodged. These scars are normally unilateral and not spirally arranged (FIG. 3 and 4, 3). They appear as a series of thickened, brown pads when viewed in profile and as rings when viewed from in front. Apparently abstraction of the conidia proceeds slowly and in a manner represented by the closing of an iris diaphragm, as indicated by the small, central ring that marks the less thickened wall near the center of the scar. The conidiophores are thus definitely geniculate and not articulately moniliform, as shown by Greville (l. c.), nodose, as shown by Corda (l. c.), nor helical, as described by Killian (5).

The conidia (FIG. 4, 2) are not seen in position usually in paraffine sections since the conidiophores are directed downward and the conidia are normally shed as they mature. They may be found occasionally, however, attached either to the tip of the conidiophore or to its lateral wall (FIG. 4, 3). The cell walls of the conidial stromata and of the basal cells of the conidiophores are brown and thick, and their content is little stained by Haidenhain's iron alum hematoxylin. The walls of the older parts of the conidiophores are brown and firm, that of the elongating tips, thin and pale yellowish brown, but they contain an abundance of stainable material.

It has been impossible at any time to secure germination of more than a small proportion of the conidia in water, and growth of the germ-tube soon ceases (FIG. 4, 4), which observation accords with that of all other investigators.

*Spermogonial stage.* During autumn, the strómatic cushions that bore the conidiophores or others that arise from the inter-cellular mycelium (FIG. 5, 7) become the stromata that produce the spermogonia. They thus arise as a lateral expansion of the conidial stromata, as shown in FIG. 2, c, d, and 5, 5, or else as separate entities. These stromata extend to the exterior from the lower leaf surface. They vary in size up to a millimeter in

diameter and consist of large, compact, thick-walled, brown cells. They are plane above with a papillate surface, each papilla marking the orifice of a spermogonium. The spermogonia are spheri-

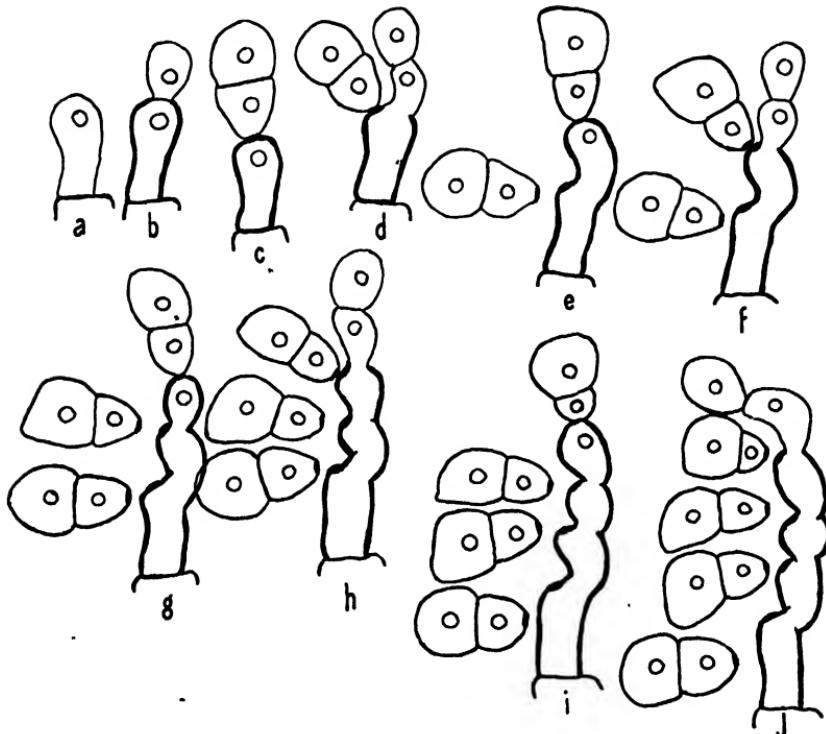


FIG. 3. Diagrams showing stages in the production of a series of conidia on a single conidiophore. a, Young, erect conidiophore; b, Young conidiophore from which a conidium is being abstricted; c, Mature conidium formed terminally; d, Elongation of the conidiophore, formation of a new conidium, and dislodging of the first-formed conidium; e, Two fully formed conidia and a lateral scar that marks the point at which the lower conidium was attached; f, g, h, i, j, Successive stages that show the repeated elongation of the conidiophore, dislodgment of the conidia, and the formation of new conidia apically.

cal to flask-shaped bodies in whose interior spermatia,  $3-5 \times 1.5-2.0 \mu$ , are formed. The spermatia arise from parietal, spermatiferous cells that are ampulliform (FIG. 5, 8). They are formed in basipetal succession in such profusion that the entire spermogonium becomes filled, and they are forced out of the orifice in pale, mucilaginous droplets. This exudate may spread over the

surface of the stroma and on drying appear as a thin crust (FIG. 2, c and 5, 5). It is this stage of the fungus, as has previously been stated, that was first described by Persoon as *Sphaeria Trifolii*. Killian (5) regards it as the pycnidial stage that is responsible for the dissemination of the fungus during fall and early winter. Bayliss-Elliott and Stanfield (1) also regard it as a pycnidial stage, and it is described by Traverso (8) as the pycnidial fungus *Placosphaeria Trifolii*. Bayliss-Elliott and Stanfield (1) state that the pycnospores germinate by budding or by the formation of germ tubes, and present evidence that pycnospores produce infection. The writer has not been able to secure their germination, and feels that there is abundant evidence that they function as spermatia.

*Ascigerous stage.* The initials of the perithecia arise within the same stromata that bear the spermogonia and their initiation is coincident with that of the spermogonia. They appear at this time as locular, spherical masses of thin-walled, deeply-staining, uninucleate cells, embedded within the stromata. Some of the cells are somewhat larger than the others and become more deeply stained with Haidenhain's iron alum hematoxylin. Such cells are interpreted to be portions of the numerous, septate archicarps. In serial sections they can be traced to trichogynes, ranging in number from a few to 30 or 40, that extend from the surface of each perithecial initial (FIG. 2, c, d, and 5, 5). The numerous trichogynes and ascogones in each perithecial fundament are similar to the condition noted in *Polystigma rubrum* by Trifonova (9). The spermatial ooze adheres in quantity to these trichogynes (FIG. 2, c). Water appears to be essential for the transfer of spermatia from the orifice of the spermogonium to the trichogynes. Unless they are brought into contact with the trichogynes further development of the perithecia is apparently impossible. This is indicated by the fact that few or no mature perithecia are developed on leaves collected when the perithecia are just beginning to form, if such leaves are kept in the laboratory and are thus removed from the possibility of becoming wetted by dews and rains. The writer's failure to recognize the necessity of water for spermatization undoubtedly accounts for the lack of perithecia in material collected, in several seasons, at times when the conidia were

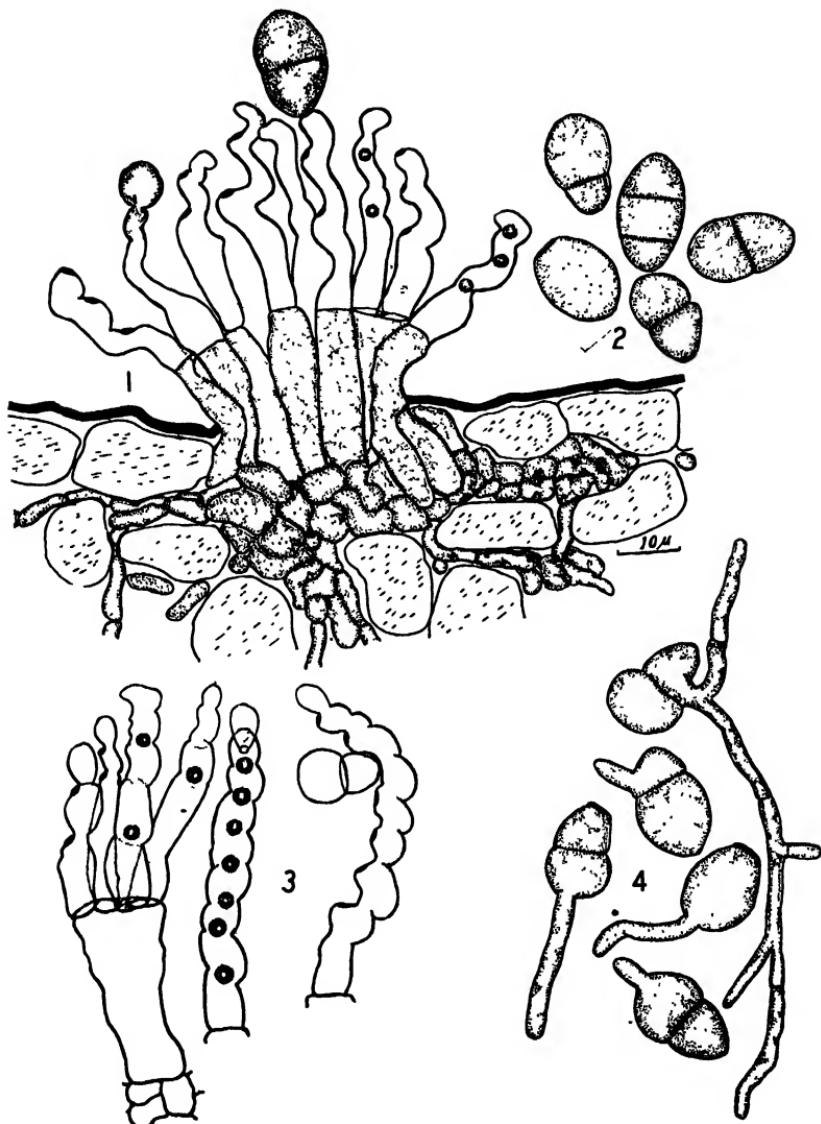


FIG. 4. 1, Conidial pustule in section showing the intercellular stroma and the foot-like cells surmounted by conidiophores. Scars from which the conidia have been detached are present; 2, Group of conidia. Most conidia are 2-celled although occasional ones are 1-celled or 3-celled; 3, Foot-like cell from a conidial pustule and conidiophores, a group of 4 conidiophores borne on one foot-cell, at the left; a row of 8 conidial scars and a young conidium being pushed aside by the elongating conidiophore, in the central figure; profile of a conidiophore that has borne 7 conidia, one still laterally attached, at the right.

abundant and when this material was stored out of doors for the winter, after it had remained in the laboratory for several weeks.

The trichogynes are collapsed by the time that the basal cells of the archicarps have become binucleate (FIG. 5, 6), indicating that the association of nuclei preparatory to fusion has occurred. The writer has not been able, however, to trace nuclear migration from the sperniata to the oögonial cells. Apparently all of the archicarps, except one, in each perithecial locule disintegrate. As this one continues its development its basal cells become multi-nucleate with pairs of closely associated nuclei (FIG. 5, 9).

A period of 4 to 6 months is required for the development of this portion of the perithecial structure. The subsequent development, including the swelling of the perithecial locule so that it protrudes above the stroma, the formation of a few to about 20 ascogenous hyphae in each perithecium, the fusion of the paired nuclei within the young ascus, the delimitation of the ascospores, and the rupture of the apex of the perithecium, may require from 4 to 6 weeks. The asci mature a few at a time, beginning with those nearest the center of the perithecium (FIG. 5, 10). The ascospores are at first elongate-elliptical and unicellular. They then become slender and septate, and at maturity are constricted at the septum and the upper cell is slightly the larger. Much of the increase in volume of the ascospores occurs in a brief period prior to their discharge (FIG. 5, 11). Expulsion normally occurs soon after maturity, as has been demonstrated by the presence of ascospores on agar plates inverted above perithecia. During germination on agar the spores first become much swollen and then a single germ tube is emitted from either cell or from both cells (FIG. 5, 12).

No portion of this fungus appears to be entirely hyaline. The stromata are brown to sepia, the cells of the perithecial locules are dilute honey yellow, the content of the asci before spore formation is honey yellow, and the ascospores are dilutedly honey yellow. Killian (5) and Bayliss-Elliott and Stanfield (1) regarded the ascospores as hyaline.

## DISCUSSION

Studies on the developmental histories of representative genera of the Dothidiales, as yet, are distinctly limited in number. Almost nothing is known regarding the initiation of perithecia and sexuality in this order. A system of classification, based upon a definite knowledge of relationships within this order, can not be devised until such studies have been made. The monographic treatment by Theissen and Sydow (7) is indispensable to any student of the Dothidiales, and assuredly it must be revised in the light of future investigations. The basic concepts that have been established by the morphologic studies of Orton (6) emphasize the close relationship of the stromatoid Sphaeriales with the Dothidiales.

If one attempts to classify the fungus under consideration according to Theissen and Sydow's keys, it is plainly one of the family Dothidiaceae. Then because of the fact that the ascospores are pigmented, it will be found to resemble most closely the genus *Systrema*, erected by Theissen and Sydow (7). The only member of this genus whose ontogenetic development has been investigated is *Systrema Ulmi* (Schleich.) Theiss. & Syd. Killian (4) found, in this organism, that one celled conidia are abstracted from palisade-like cells at the surface of the stromata. He found within the young stroma what he interpreted to be a male and a female cell whose nuclei became associated by the migration of the male nuclei to the cell containing the female nuclei. Then on entering the ascogenous hyphae these pairs of nuclei united. This type of development is most certainly very different from that exhibited by the sooty blotch fungus of clover, and there seems to be little grounds for regarding it as of the same generic type. It should also be recalled that *Polythrincium* has remained for over a 100 years a monotypic genus<sup>2</sup> and that its characters are so distinctive that there appears little reason for disregarding them in classifying the pathogen on clovers. It is, therefore, proposed to erect for the clover fungus the new generic name *Cymadothea*, derived from words that refer to the undulate character of the conidio-

<sup>2</sup> Another species, *Polythrincium Shirainum* P. Henn. (Bot. Jahrbüch. Syst. Pflanzengesch. Pflanzengeog. 37: 165, 1905) has been described as occurring, in Japan, on *Cercidophyllum japonicum* S. & Z.

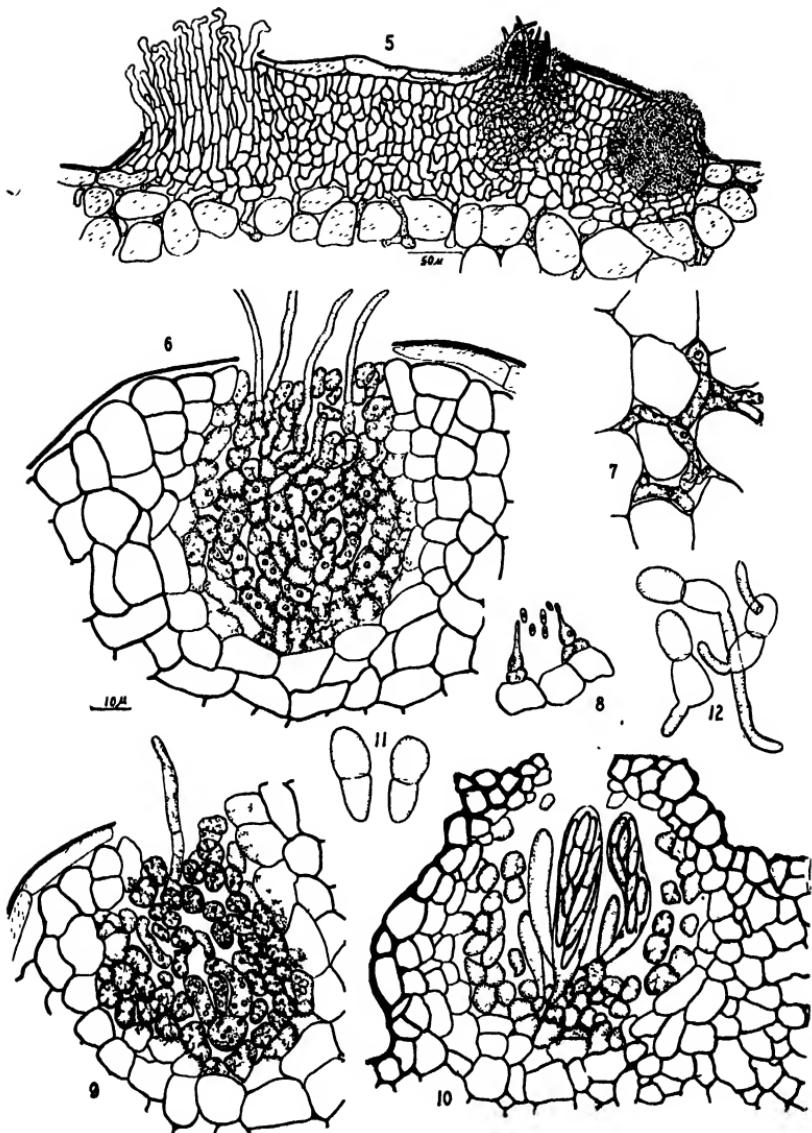


FIG. 5. 5, Section of a stroma showing, within one stroma, an old conidal pustule, a perithecial initial, and a spermogonium. The spermatia have welled out of the spermogonium and piled up among the projecting trichogynes; 6, Perithecial stroma in which the nuclei have become paired in the ascogones; 7, Intercellular hyphae within the tissue of green clover leaves; 8, Spermatiferous cells and spermatia from the parietal portion of the spermogonium; 9, Young perithecial stroma in which the ascogonium contains several pairs of nuclei that have arisen by conjugate division; 10, Perithecium of *Cymadothea Trifoli* that has irregularly opened and contains asci in different stages of maturity; 11, Mature ascospores immediately after expulsion onto agar plates; 12, Germinating ascospores.

phores of the conidial stage, and of the stromatic, dothidioid character of the perithecial stage.

**Cymadothea** gen. nov. (Etym. *κυμα*, wavy or undulate, and *δοθικήν*, swelling or stromatoid outgrowth.)

Stroma innatum, erumpens, dothideoideum, brunneum; peritheciis in stromatibus oriundis, irregulariter dehiscentibus, sphericis vel ampulliformibus; ascis clavatis, aparaphysatis, octosporis; sporis didymis, subhyalinis vel helvis. Status conidicus *Polythrincium* est.

**Cymadothea Trifolii** (Pers.) comb. nov.

Syn. *Sphaeria Trifolii* Pers. Syn. Fung. 30. 1801.

*Polythrincium Trifolii* Schm. & Kze. Deutschl. Schwaemme 5: 5. 1816.

*Dothidea Trifolii* (Pers.) Fries, Summa Veg. Scand. 387. 1849.

*Phyllachora Trifolii* (Pers.) Fuckel, Symb. Myc. 218. 1869.

*Placosphaeria Trifolii* (Pers.) Trav. Ann. Myc. 1: 130. 1903.

*Polythrincium Trifolii* var. *platensis* Speg. Anal. Mus. Nac. Buenos Aires III. 13: 437. 1911.

*Phyllachora umbilicata* Theiss. & Syd. Ann. Myc. 13: 510. 1915.

*Plowrightia Trifolii* (Pers.) Killian, Rev. Path. Entom. Agr. 10: 219. 1923.

*Dothidella Trifolii* (Pers.) Bayliss-Elliott & Stanfield, Trans. Brit. Myc. Soc. 9: 226-227. 1924.

Stromatibus pseudoparenchymaticis, brunneis vel atrofuscis; peritheciis in spermatiferis iisdem vel similibus evolutis, ampulliformibus; ascis clavatis, octosporis, aparaphysatis; sporis subhyalinis vel dilute helvis, 1-septatis, loculis inaequalibus,  $20-26 \times 8-9 \mu$ .

Hab. in pagina adverso foliorum *Trifolii* emortui, in verno tempore.

Status spermogonicus: stromatibus in autumno efformantis, hypophyllis, pseudoparenchymaticis, ambitu irregulariter circularibus, brunneis v. atrofuscis, intus uni-pluri-loculatis, contextu loculorum parenchymatico et helvis; spermatiis ellipsoideis, saepe curvatis,  $3-5 \times 1.5-2.0 \mu$  in guttulis vel cirris exsudatis; spermatiophoris ampulliformibus. Hab. in foliis vivis v. dejectis *Trifolii*, socio *Polythrincio Trifolii*.

Status conidicus: Maculis fuscentibus; conidiophoris hypophyllis, fasciculatim erumpentibus, punctiformibus, deinde coalitis, ex mycelio in parenchymate folii oriundis, tortuosis vel exquisite undulatis, geniculatis, fuscis,

aseptatis, apice conidia gerentibus, posterius egredientibus et conidia alia gerentibus; conidiis ovatis, brunneolis, inaequaliter bicellularibus, inferiore cellula minor,  $20-24 \times 11-15 \mu$ . Hab. in foliis viridis *Trifolii* sps.

The microscopic preparations upon which this study has been based have been deposited in the Farlow Herbarium, Harvard University.

#### SUMMARY

This study deals with the morphology of the sooty blotch fungus of *Trifolium*, which has a conidial stage, a spermogonial stage, and a perithecial stage in its cycle of development.

The undulate character of the conidiophores is the result of sympodial branching. Each conidiophore produces a series of conidia. After each conidium is delimited the conidiophore elongates.

The spermogonia and perithecial fundaments are formed coincidentally within the same stroma, sometimes in separate stromata, in autumn. Each is locular.

The spermogonial stage functions in the production of spermatia that apparently are essential to the subsequent development of the perithecia.

The perithecial stage matures in spring within locules of a dothidiaceous stroma.

The evidence in hand indicates that all parts of the fungus are pigmented with some tint or shade of brown.

Several names have been applied to each of the stages in the developmental cycle of the organism. It is herein regarded as the type of a new genus and is given the name *Cymadothea Trifolii* (Pers.).

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DURHAM, N. C.

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# A LITTLE KNOWN PECAN FUNGUS

VERA K. CHARLES

(WITH 2 TEXT FIGURES)

It frequently happens that a fungus will appear and be described and then be forgotten or pass unobserved for a long period of time. This appears to be the history of an interesting fungus recently observed on pecan leaves from Texas.<sup>1</sup> A search through the mycological collections of the Bureau of Plant Industry disclosed one specimen of this fungus collected in 1900 on pecan leaves, but erroneously determined as *Microstroma Juglandis* (Bereng.) Sacc. Another specimen in the Collections was collected by L. E. Miles in 1920 at DeSoto, Mississippi, on leaves of *Quercus* sp.

The fungus on pecan is very minute and appears as snow-white tufts on the lower surface of the leaves (TEXT FIG. 1, A).

A microscopic examination of the fungus on pecan showed it to be closely related to what Peck (6) described as the conidial stage of his *Ascomycetella quercina* (TEXT FIG. 2, B). The striking characters of the fungus are the tiered or fan-shaped, superimposed arrangement of the bundles of hyphae and the barrel-shaped cluster of closely adhering spores. Other details are given in the following description by Peck:

"Hyphae tufted, colorless, compound, composed of superimposed, somewhat obconic masses of obovate cells placed side by side and bearing on the upper and outer margin of the masses verticels of conidia; conidia oblong or subcylindrical, slightly curved, colorless, .0005-.0006 of an inch long, .00016-.0002 of an inch broad, produced in subelliptical tufts or masses, .0005-.0006 of an inch long and about .0005 of an inch broad, each tuft composed of seven occasionally six conidia, compactly placed side by side in a circle, and forming a cylinder around a central one." Peck, l. c.

<sup>1</sup> This material was received from J. B. Demaree in November, 1933, who collected it along the Colorado River at Utley, Texas.

Careful examination of Peck's material and that on the pecan leaves showed the group of spores to consist of eight instead of six spores. Peck's drawings also indicate this number.

Peck also described what he considered a new ascogenous genus, *Ascomycetella*, which was associated with this conidial form, and

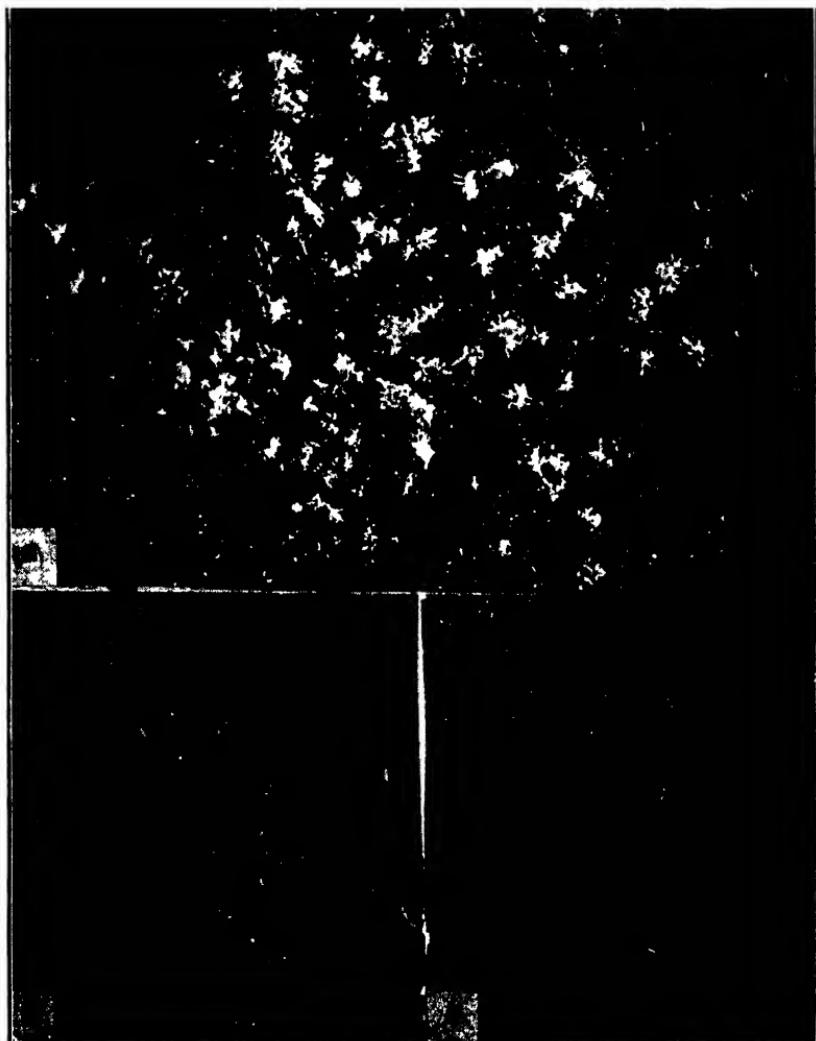


FIG. 1. a, Lower surface of a pecan leaf showing *Articularia quercina* (Peck) Höhn. var. *minor*.  $\times 12$ ; b, (From Desmaziere's Pl. Crypt. Fr., fasc. 19, no. 929, 1838).  $\times 425$ ; c, *Articularia quercina* (Peck) Höhn. var. *minor*.  $\times 425$ .

which he took to be its perfect stage. The genetic relationship of these two fungi was not demonstrated by culture work and the inference is that the supposed relationship was based only on the close association of the two fungi. No ascogenous fungus was found to be present on either of the two collections on pecans from Texas, the first in 1900 or the second in 1933.

The next study of this fungus seems to be that of von Höhnel (3) in 1909 who described a new genus to care for Peck's hypothetical conidial stage, and which he called *Articularia* (TEXT FIG. 2, n). The following is a translation of the description of this fungus as given by von Höhnel:

#### *Articularia* von Höhnel

Sterile hyphae consisting of a loose hyaline tubercular-like tissue. Fruiting hyphae simple, consisting of obconical tiers or sections, each tier composed of a whorl of obconical, truncate, single-celled conidiophores, each bearing a barrel-shaped cluster of 8 hyaline, parallel spores.

Von Höhnel assigned this genus to Mucedineae, but in concluding the discussion of the genus stated it might also be placed with the Tuberculariaceae.

In the same work von Höhnel (l. c.) described a second genus which he called *Articulariella* (TEXT FIG. 2, c) and which he considered to be the conidial stage of Ellis and Martin's (2) *Ascomycetella aurantiaca*, described in 1885. The following is a translation of the description of this genus and species as interpreted by von Höhnel:

#### *Articulariella* von Höhnel

Structure as in *Articularia* but the whorled side branches parallel with the axis, the ends enlarged or rounded and bearing a crown of 5-8 one-celled, oblong-elliptical spores which become separate.

#### *A. aurantiaca* (Ellis & Mart.) von Höhnel

Fungus white, sterile hyphae forming a tubercular tissue from which the long, fragile conidiophores arise and from which the asci later develop. The column of conidiophores consists of about 10-12 one-celled, hyaline, parallel hyphae,  $20-40 \times 2-3 \mu$  in size, rounded at the apex and bearing a crown of 5-8 1-celled, elliptical-

oblong, hyaline spores, the latter straight, narrower at both ends and  $6-9 \times 1.5-2 \mu$  in size.

Comparing this description with the original description by Ellis and Martin (l. c.), the general characters are found to be the same but the measurements of the spores are smaller, von Höhnel's measurements being  $6-9 \times 1.5-2 \mu$  and those of Ellis and Martin  $5-7 \times 2-3 \mu$ . The specimen of *A. aurantiaca* Ellis & Mart. examined during the course of this study was that of Ellis and Everhart, North American Fungi 2068, 1883. An examination of this specimen showed the spore measurements to agree with those given by Ellis and Martin in their description of the fungus. In this instance, as in the case of *Articularia*, Peck's conidial stage, the genetic relationship was only inferred from the close association of the imperfect and ascogenous fungi and not demonstrated by culture work.

According to von Höhnel, *Articularia* differs from *Articulariella* in having closely adhering barrel-shaped bundles of cylindrical spores, eight in number, while in *Articulariella* the conidiophores are enlarged at the apex and bear a crown of  $5-8 \mu$  elongate, rod-shaped spores,  $6-9 \times 1.5-2 \mu$ , with somewhat obtuse, narrower ends. However, as previously stated, a study of Ellis and Martin's material showed the spores to range from  $6-7 \mu$  in length. Von Höhnel considered this fungus his *Articulariella aurantiaca*, described by Ellis and Martin as the conidial stage of their *Ascomycetella aurantiaca*, to be the conidial stage of *Leptophysma aurantiaca* (Ellis & Martin) Sacc.

In discussing *Articularia* von Höhnel called attention to what he considered a nearly related fungus described by Corda as *Fusisporium uncigera*. In 1884, Saccardo (7) established the genus *Uncigera* for this fungus and called it *Uncigera Cordae* Sacc. Later, von Höhnel in discussing this species states that according to the rules of nomenclature it should be designated *Uncigera uncigerum* (Corda). The description and illustration of this specimen show it to have little resemblance to the fungus under consideration and may be eliminated from the discussion.

In 1925, sixteen years after von Höhnel's description of the two genera, *Articularia* and *Articulariella*, Arnaud (1) in his paper on "Les Asterinées" claimed that the two genera *Articularia* and

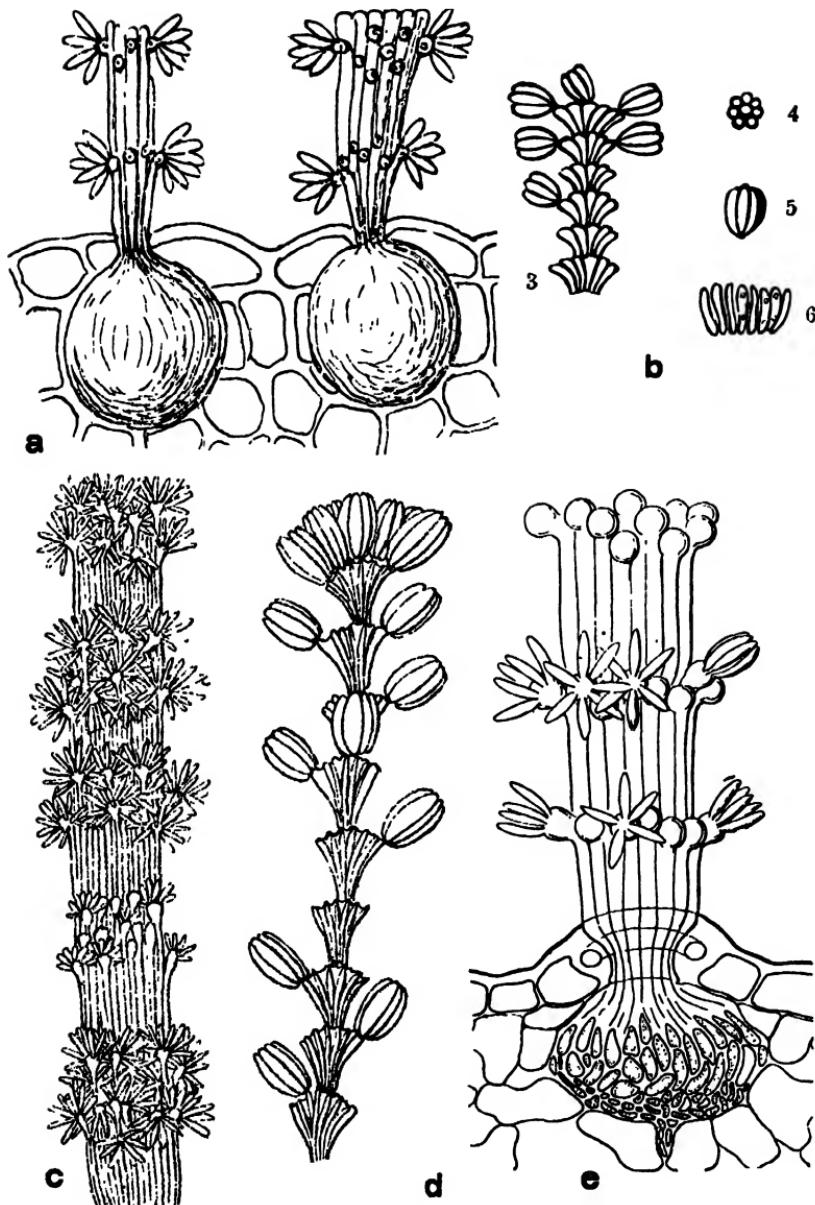


FIG. 2. a, *Helostroma album* (Desm.) Pat. After Patouillard; b, Conidial stage of *Ascomycetella quericina* Peck. After Peck. c, *Articulariella aurantiaca* (Ellis & Mart.) Höhn. After von Höhnel. d, *Articularia quericina* (Peck) Höhn. - After von Höhnel. e, *Helostroma album* (Desm.) Pat. From Desmaziere's Pl. Crypt. Fr., fasc. 19, no. 929 (1838), under the name *Fusisporium album*. After Arnaud.

*Articulariella* are identical and referred them to *Helostroma album* (Desm.) Pat. (TEXT FIG. 2, A), a genus established by Patouillard (5) in 1902 to better describe *Fusisporium album* Desm.

The following is a free translation of Patouillard's description of *Helostroma* and shows a close agreement with von Höhnel's *Articulariella*:

Mycelium forming small, white, stromatic masses in the spongy parenchyma of the leaves, especially beneath the stroma. The tubercles consist of a filamentous structure about  $25\ \mu$  and develop an erect, hyaline, cylindrical column emerging from the stroma,  $25-35 \times 7-10\ \mu$ , and truncate at the summit. The column is composed of colorless, parallel, cylindrical filaments  $2-3\ \mu$  in diameter, crowded, unequally elongated and bearing the conidia at the apex on a short, lateral obtuse enlargement. The enlarged apices of the conidiophores appear as a crown about the column, sometimes a second crown develops towards the middle of the column. Six to seven, colorless, straight, elliptical conidia, measuring  $5-6 \times 2-3\ \mu$ , are borne on each conidiophore. Patouillard stated that Saccardo transferred this species to the genus *Microstroma*, near *Microstroma Juglandis* but from Saccardo's description of the monosporous, clavate basidia, Patouillard inferred that Saccardo must have had an entirely different species. Maire (4) also remarks that Saccardo's specimen was considered monosporous because all but one spore had fallen away. An examination of Saccardo's material contained in the Mycological Collections showed it to be characteristic of *Helostroma album* (Desm.) Pat. A microscopic examination of specimens of well known exsiccati and miscellaneous specimens in the Mycological Collections of the Bureau of Plant Industry, labelled *Microstroma album* (Desm.) Sacc., showed them to agree closely with Patouillard's genus *Helostroma*. Patouillard reported this fungus as parasitic on leaves of various oaks.

From the description and illustration of *Helostroma* by Patouillard and an examination of Desmaziere's material, it would seem that *Articulariella* von Höhnel is synonymous with *Helostroma album* (Desm.) Pat. Although von Höhnel's illustrations certainly show remarkable differences between the two fungi, Arnaud (l. c.) says that the apparent differences in the illustrations are

due to the fact that one figure was described from a surface view and the other a lateral view. This author made no mention of the difference in the shape of the conidiophores or in the size and shape of the spores. The article is accompanied by a plate showing a fragment of a leaf of *Quercus* from Rabenhorst-Winter, *Fungi Europæi* 3040, which is Peck's material. The illustrations show only the detached agglutinated spores of Peck's so-called conidial form of *Ascomycetella quercina* or von Höhnel's *Articularia*. Arnaud gives no description nor illustration of the conidiophores or mention of the difference in the size and shape of the conidia. Probably Arnaud's specimen of Peck's material did not show the spores attached. The illustration shows the conidial form in close association with the ascogenous form, termed by Peck *Ascomycetella quercina*. In addition to Arnaud's plate there is a drawing of considerably larger magnification of *Fusisporium album* Desm. (TEXT FIG. 2, E) (Desmaziere's Pl. Crypt. Fr., fasc. 19, No. 929, 1838). This drawing agrees with our observations and interpretation of Desmaziere's material, but we can not consider it identical with Peck's material, that is his so-called conidial stage of *Ascomycetella quercina* or with our fungus on pecan. However, it does agree with our understanding of Patouillard's *Helostroma album* (Desm.), the fungus commonly known as *Microstroma album* (Desm.) Sacc. It appears that Saccardo's use of the generic name *Microstroma* has been followed by many mycologists although the generic characters of the genus do not satisfactorily describe this species.

During the present study many specimens of *Microstroma album* in well known sets of exsiccati and miscellaneous collections were examined and found to be typical of Desmaziere's *Fusisporium album*.

Arnaud further stated that *Articularia* and *Articulariella* do not represent the conidial stage of the ascogenous form, *Ascomycetella quercina*, described by Peck, but are two different fungi and that the ascogenous form is *Cookella microscopica* Sacc. present as a parasite on the *Helostroma*.

In view of the fact that there is a question of the genetic relationship of this fungus with an ascogenous form and without opportunity for field observations, it seems best to retain von

Höhnel's generic name, *Articularia*, until the life history of the fungus is definitely known. That this fungus may be classed with the Mucedineae as suggested by von Höhnel seems logical although it has characters which suggest the Tuberculariaceae, a reference also suggested by von Höhnel.

The material of *Articularia quercina* (Peck) von Höhnel available for study consisted of five specimens collected by F. S. Earle at Cobden, Ill., in 1882 and 1883 on *Quercus tinctoria*, a collection of G. H. Demetrio on *Carya alba* collected at Perryville, Mo., September, 1883, and issued by Rabenhorst-Winter (Fungi Eu. 3388b) as *Microstroma leucosporum* (Mont.), and another collection by L. E. Miles at De Sota, Miss., Sept. 21, 1920 on *Quercus* sp.

The two fungi, the one described on oak, *Articularia quercina* (Peck) von Höhnel, and the form on pecan, are very similar in their general morphology but the spore measurements are sufficiently different to deserve a varietal name, accordingly the fungus on pecan will be designated *Articularia quercina* var. *minor* with the following description:

*Articularia quercina* (Peck) von Höhnel var. *minor* var. nov.

Caespitulis candidis, flocculosis; conidiophoris 0.5 mm. alt., articulis 12–16  $\mu$ ; conidiis oblongis-fusoideis, hyalinis, octonis in corpus elliptico-doliiforme 6–8  $\times$  2.5–3  $\mu$  coalescentibus.

Fungus caespitose, effuse, white, hyphae forming closely adhering parallel columns, spuriously stilbaceous, compact superimposed, composed of obovate cells, placed side by side and bearing on the upper and outer margins 8 spores; spores oblong to subcylindrical, slightly curved, colorless, 6–8  $\times$  2.5–3  $\mu$ , agglutinated in bundles.

Habitat on the lower surface of living leaves of *Hicoria illinoensis*.

Type: Utley, Texas, collected by J. B. Demaree, 1933. Deposited in Mycological Collections, Bureau of Plant Industry. A second collection by R. H. Price at College Station, Texas, Sept. 12, 1900.

SUMMARY

From the present study of the fungi, variously known as *Fusisporium album* Desm., *Helostroma album* (Desm.) Pat., *Articu-*

*laria quercina* (Peck) von Höhnel, *Articulariella aurantiaca* (Ellis & Martin) von Höhnel, *Ascomycetella quercina* Peck, *A. aurantiaca* Ellis & Martin, *Microstroma alba* (Desm.) Sacc., it is believed that *Articulariella aurantiaca* (Ellis & Martin) von Höhnel is synonymous with *Fusisporium album* Desm., *Helostroma album* (Desm.) Pat. and *Microstroma album* (Desm.) Sacc., but that *Articularia quercina* (Peck) von Höhnel is another fungus described as the conidial stage of Peck's *Ascomycetella quercina*, and in general, the fungus present on pecans.

Because of the difference in the size of the fungus on oak and the one on pecan, it seems best to consider the form on pecan a variety and designate it *Articularia quercina* (Peck) von Höhnel var. *minor*.

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## NOTES AND BRIEF ARTICLES

### AN UNWELCOME GUEST

The May number of the *Gardeners' Chronicle* for 1934 contains an article by E. Holmes-Smith, Advisory Mycologist, North-Western Province, Manchester University, England, on "A playing-fields fungus menace." He states that large sums of money had been spent by the National Playing Fields Association to convert areas which had formerly been dumping grounds for all kinds of debris into playing fields. Tennis courts constructed on such grounds have been entirely ruined through the upheaval of certain fungi, apparently *Coprinus comatus*, which has insisted on intruding itself over the entire field in huge clumps.



FIG. 1. Tennis court destroyed by *Coprinus*.

Since the article was published as a warning it seems not out of place to repeat the warning here. In doing so the writer is in-

debted to E. Holmes-Smith for an original photograph of the ruined area which is reproduced here with his permission.

In *MYCOLOGIA* 25: 150, 1933, a note was published by A. R. Bechtel on the "Lifting power of a mushroom." Keeping this in mind we may have some idea of the amount of damage that may be done by the eruption of a tennis court through the agency of these organisms.—F. J. SEAVER.

#### ARE LIVING SPORES TO BE FOUND OVER THE OCEAN?

Microörganisms are known to be rare over high mountains or large bodies of water. The writer, however, has not noted reports of exposure of Petri plates from a ship crossing the ocean. Such exposures were made from the SS. *Alaunia*, which left Montreal about noon May 25, 1934, and sailed *via* the St. Lawrence route south of Newfoundland to Plymouth, Havre and London. London was reached at noon June 4. The plates were exposed morning and evening, by holding them into the wind by hand. During the first three and the last two days of the trip, land was in sight and exposures of one or two minutes were made. During the intervening five days over the ocean, the exposures were for  $2\frac{1}{2}$  or 3 minutes. Eighteen plates in all were used, half being of Czapek's agar, half of potato-sucrose. The plates were then taken to the Imperial Mycological Institute, Kew, where they were incubated further and examined. The results showed that the precautions taken to avoid contamination were effective.

In the St. Lawrence River and Gulf, three or four microörganisms, about equally fungi and bacteria, were obtained on each exposure of two minutes. Cape Race, Newfoundland, was passed in a fog, and twenty bacteria developed from a  $2\frac{1}{2}$  minute exposure there at 8 A.M., May 28. With the last land astern, no microorganism was obtained for  $4\frac{1}{2}$  days, except for 3 colonies of bacteria on one plate exposed in mid-ocean on May 30; these perhaps were carried on an eddy of air from the ship. On June 1st at 8:45 P.M. the ship was just S. W. of Ireland with a wind blowing from the N. E.: 19 colonies of bacteria developed. The following morning an exposure south of the Irish coast yielded four fungi, one of which was *Botrytis cinerea* and another, appropriately enough, seemed to be *Phoma hibernica*. In the English

Channel 2 to 5 each of fungi and bacteria were obtained on each exposure of one minute, except for one blank in mid-channel.

The results obtained convinced the writer that microorganisms are so scarce over the ocean as to necessitate special arrangements for long exposures of plates and slides.—G. R. BISBY.

#### MUSHROOM POISONING DUE TO AMANITA COTHURNATA

In Ontario from June to September, 1934, the weather was drier and hotter than usual. Early to mid-September rains brought on a good crop of delayed fleshy fungi.

*Amanita cothurnata* Atk. was one species that appeared more plentiful than usual. On a ridge between two ravines, in a thin woods chiefly of beech, I observed a "fairy ring" of this agaric 18 feet in diameter. The plants were strictly limited to the circumference of the circle and along this line I counted 65 of them. The general appearance of the ring was white. The cuticle of the different pilei showed a range of color from very pale cream to apricot buff (R.); every part below it was pure white. One cap had become inverted without cracking and retained what must have been about a tea-cupful of rain water. I brought home the doubtfully largest specimen for further study. It measured 26 cm. over the marginally striated cap; the hollow stem reached 20.2 cm. above the base of the volva and bore a correspondingly ample annulus; the volva with a circumference of 18 cm. terminated above in a shallow but well marked, flaring roll. The flesh was odorless and the taste pleasant enough for a raw mushroom.

*Lepiota naucina* Fries was also plentiful and under the somewhat dangerous name "white mushrooms" was offered for sale on the market.

On Sunday, Oct. 7th, George Magorka of London, Ont., collected what he called "brown mushrooms"—*Hypholoma sublateritium*—and "white mushrooms"—*Amanita cothurnata*—in quantities to eat and to dry for winter use. In the evening about an equal division of the two kinds was prepared and boiled from 6 to 9 P.M., then seasoned, fried in cream, eaten and considered delicious. Supper over, the family consisting of the parents and a daughter retired in comfort for the night. But in the words of a poet ".midnight saw another sight"; in less than three hours

they were all seized with serious illness. The father's irregular attempts to run to and from a physician's office attracted the attention of a policeman who for a time badly misjudged the situation. The doctor quickly responded to the call and assisted nature's efforts to get rid of the poison with warm mustard draughts and apomorphine and as quickly as possible had the patients removed to the hospital. There stomach irrigation, atropin injection and castor-oil purgation completed the treatment. Recovery was rapid.

In comparison with muscaria poisoning which it resembled in several respects there was no noticeable pupillary contraction or salivation. Promptitude of treatment should not be overlooked in reaching conclusions respecting the virulence of the toxicity of the cothurnate Amanita.

It was convenient for me to explore the locality of the collections and to examine the uncooked material. There is no reason to doubt that the two species named were the only ones involved. The family on feeling ill immediately blamed the "white mushrooms" because they had several times previously prepared and eaten the brown ones with no ill effects.—JOHN DEARNESS.

#### AN ADIRONDACK MYXOMYCETE

During the foray of the Mycological Society of America at Seventh Lake, Adirondack Mountains, New York, from August 21 to 24, 1934, Mr. Rispaud, associate of the writer, collected a Myxomycete of the genus *Lamproderma*, that later study would not reconcile with any species now recognized in the genus. A portion of the collection was sent to Miss G. Lister in England, who writes that it is similar to the type of *Enerthenema muscorum* Léveillé from New Granada (B.M. 1032), and referred to in the Monograph, ed. 3, p. 154, where it was provisionally placed with *Lamproderma scintillans* (Berk. & Br.) Morgan; and that it seems to be a persistent form.

The Adirondack specimens are somewhat like *L. scintillans*, but the stalks are shorter and stouter, and the color of the sporangia is darker when the walls are removed. The capillitium is dark throughout, not pale at the base as in *L. scintillans*. The spores, 10 to 12  $\mu$  in diameter, are beyond the range of the latter species

in size, and are coarsely marked with dark, strong spines. It is not *L. scintillans*, nor any other recognized *Lamproderma*, and is evidently Léveillé's form which should be regarded as a distinct species.

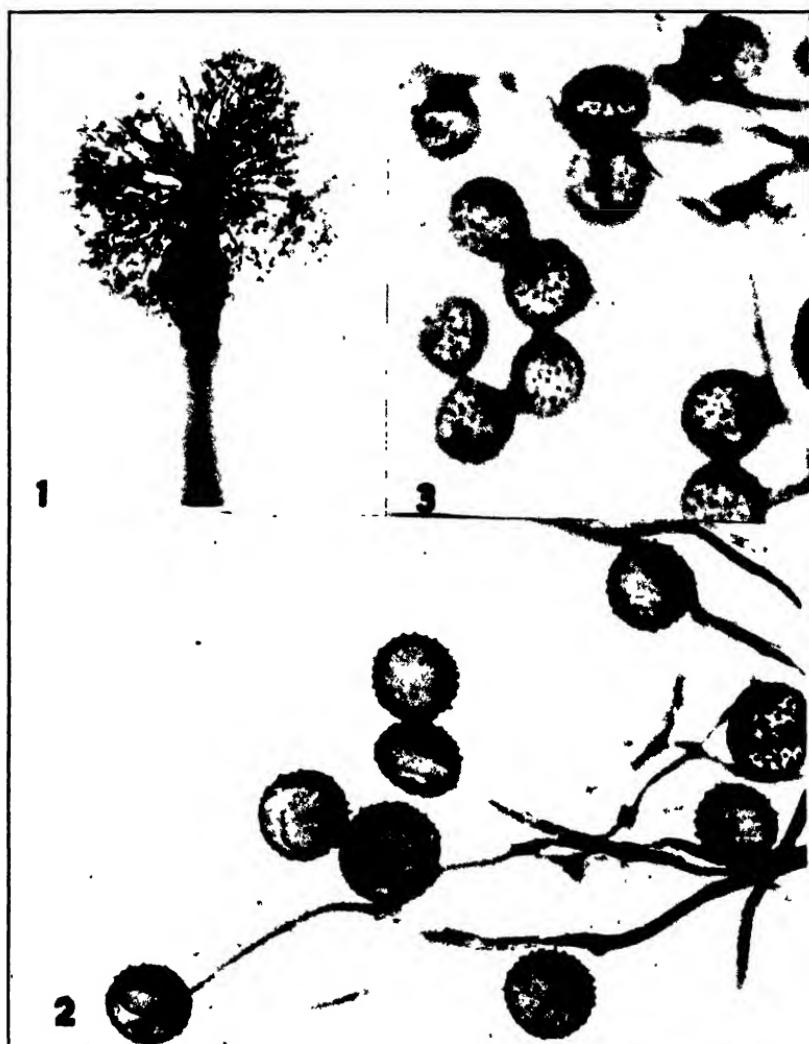


FIG. 1-3. *Lamproderma muscorum*. 1, Capillitium,  $\times 100$ ; 2, Spores, focussed on periphery,  $\times 1000$ ; 3, Spores, focussed on top,  $\times 1000$ .

My thanks are due to Miss Lister for her aid and advice in relation to this form.

**Lamproderma muscorum** (Lév.) Hagelstein, comb. nov.*Enerthenema muscorum* Lév. Ann. Sci. Nat. IV. 20: 289. 1863.

Plasmodium? Total height .6 to 1. mm. Sporangia scattered, globose, stalked, erect, 0.3 to 0.5 mm. diam., blue or bronze, iridescent; sporangial wall thin, membranous, somewhat persistent at the base. Stalk subulate-setaceous, black, shining, about one-half the total height, rising from a circular, purple-brown hypothallus. Columella thick, tapering slightly to the obtuse end, extending half-way into the sporangium. Capillitium dense, of rigid threads, radiating in all directions from the apex of the columella, dichotomously forking and branching, purple-brown throughout from base to tips. Spores violet-brown, 10 to 12  $\mu$  diam., marked with large, sharp, scattered spines.

On leaves. North of Seventh Lake, Adirondack Mountains, New York. August 24, 1934.—ROBERT HAGELSTEIN.

MINEOLA, N. Y.

In a previous issue of *Mycologia* (25 (5) Sept.–Oct. 1933) under the heading of "Abnormal spores of some *Ganoderma*" I recorded the presence of "gasterospores" in some few specimens of *Ganoderma lucidum* and *Ganoderma applanatum*. Recently, in September and October 1934, I had the opportunity of subjecting four living specimens of *Ganoderma lucidum* attached to their substrata (pieces of stem-bark and roots) to continuous showers of running water from a tap in the laboratory for a week on different dates. In each case it was found that "gasterospores" were formed abundantly within the pore-tubes. I wonder if this might explain how "gasterospores" are formed in nature in those specimens which are directly exposed to continuous showers of rain for successive days.—S. R. BOSE

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**PHYTOPATHOLOGICAL CLASSICS**

Under the above title the American Phytopathological Society is publishing a series of papers four of which have already been issued as follows:

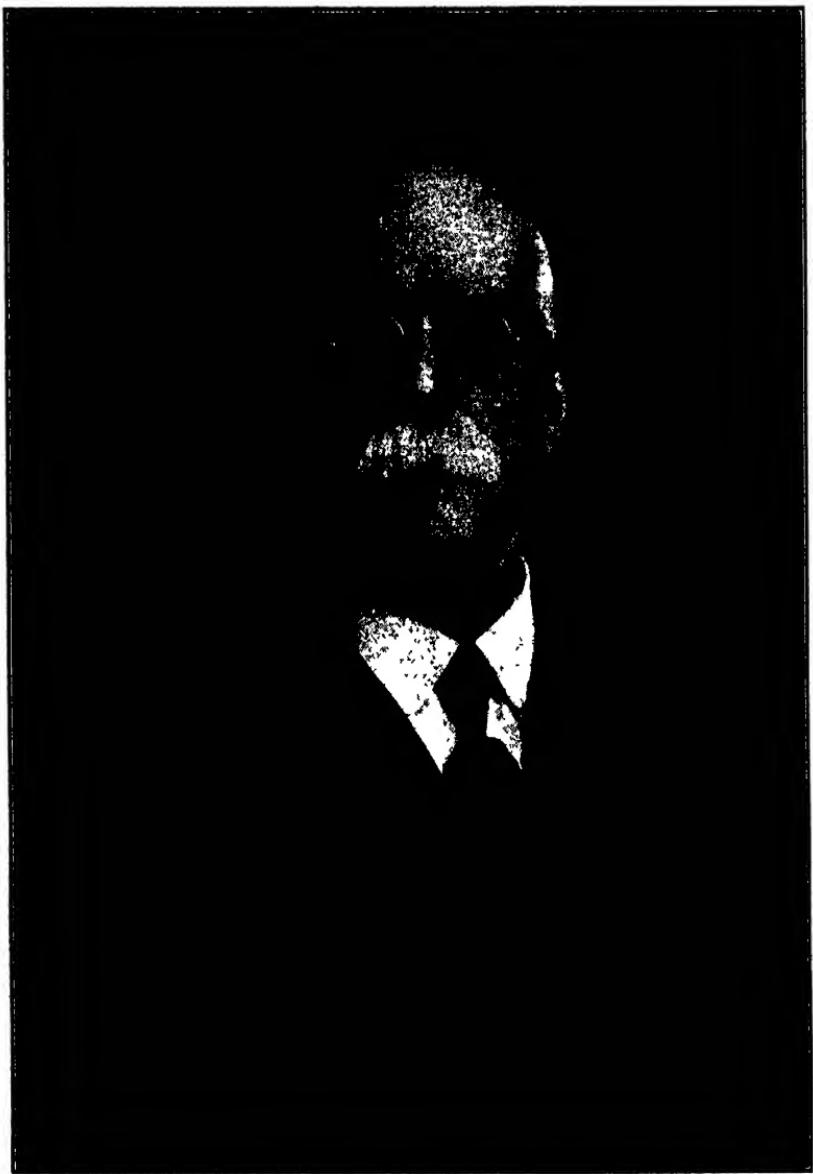
Classic No. 1. Fabricius. Attempt at a dissertation on the diseases of plants. 1774. Translated from the Danish by Mrs. Margaret Kølpin Ravn.

Classic No. 2. Fontana. Observations on the rust of grain.  
1767. Translated from the Italian by P. P. Pirone.

Classic No. 3. Millardet. The discovery of Bordeaux mixture. Three papers. Translated from the French by F. J. Schneiderhan.

Classic No. 4. Woronin. *Plasmodiophora brassicae*, the cause of cabbage hernia. 1878. Translated from the German by Charles Chupp.

These may be had at fifty cents each for the first three and seventy-five cents for the fourth, or all four, if ordered at the same time, for two dollars. Applications should be made to H. H. Whetzel, Cornell University, Ithaca, New York. Other numbers are in course of publication.



ELAM BARTHOLOMEW

# MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

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No. 2

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## ELAM BARTHOLOMEW

E. T. BARTHOLOMEW<sup>1</sup>

(WITH PORTRAIT)

Elam Bartholomew was born at Strasburg, Pennsylvania, June 9, 1852. He was later taken by his parents to Ohio and then to a farm near Farmington, Illinois, where he completed the district school and grew to early manhood. When Dr. Bartholomew decided to teach school he found that a general knowledge of botany, which he had never studied, was a prerequisite to the obtaining of a teacher's certificate in Illinois. Accordingly, he purchased a copy of *Gray's Lessons in Botany* and had soon passed the examinations in botany as well as in the other required subjects. Following the close of his term of school in the spring of 1874 he decided to "go west." Arriving in northwest Kansas he at once homesteaded a farm in the Bow Creek Valley, near Stockton. He lived on this farm for 55 years. In 1929 he moved to the Fort Hays Kansas State College at Hays, Kansas, where he became curator of the mycological herbarium. He held this position until the time of his death, November 18, 1934.

The subject of this sketch did not attend school except as mentioned in the preceding paragraph, but the purchase of *Gray's Lessons in Botany* in 1873 started him in a line of work that gradually occupied more and more of his time until it became his life work.

<sup>1</sup> University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif.

[*MYCOLOGIA* for January-February (27: 1-89) was issued February 1, 1935]

Soon after his arrival in Kansas he began to collect, preserve, and classify flowering plants. By 1885 he had in his herbarium a specimen of every phanerogam growing in that part of the state. As head of a rapidly enlarging family and as a farmer he was very busy, but at every spare moment during the day and until late hours at night, he was always found with a book in his hand. Without any assistance he acquainted himself not only with books on phanerogamic botany but with the Latin that was needed for his scientific work. He tutored several students in the latter subject so that they could pass the entrance examination admitting them to the Kansas State Agricultural College.

One day in July, 1885, Dr. Bartholomew, like Cincinnatus of old, was plowing in his field when W. A. Kellerman, then professor of botany at the Kansas State Agricultural College, came to see him. The two had never met but had corresponded much concerning phanerogamic matters. After some time Dr. Kellerman stooped down, pulled a leaf from a weed, and straightening up said, "Bartholomew, why don't you study something that is really interesting? Look at this leaf." It was an *Amaranthus* leaf and was covered with white pustules (*Albugo*) on the lower surface. Bartholomew did become interested and many times in later years as he developed his mycological herbarium, he was heard to remark: "The plucking of that leaf by Professor Kellerman from a weed in my cornfield marked a turning point in my life."

In 1887 Dr. Bartholomew's mycological herbarium consisted of only 31 labeled specimens, but at the time of his death the number had increased to about 38,000,<sup>2</sup> being composed of approximately 850 Agarics, 1,300 Polypores, 14,000 Rusts, 1,200 Smuts, 7,450 miscellaneous saprophytes, and 13,000 other forms. In addition to North American fungi the herbarium contains many hundreds of specimens from Sydow's *Fungi Exotici*, *Phycomycetes*, *Mycotheca Germanica*, *Uredineen*, and *Ustilagineen*; Petrak's *Czechoslovakian fungi*; Vestergren's *Swedish fungi*; *Fungi Europaei*; Cuba, The Philippines, Malaya, and from many other foreign sources.

<sup>2</sup> Figures are for 1931. Since that time the number has been increased to about 40,000.

In 1901, Dr. Bartholomew became editor and publisher of Ellis and Everhart's *Fungi Columbiani*, composed of specimens of North American fungi of all kinds. He continued this publication until 1917, having published 36 centuries of this work during the years from 1901 to 1917. In 1911 he began the publication of a new work called *North American Uredinales*. This was somewhat similar to the preceding but included only the rust fungi of North America. He published 35 centuries of this new work. The publication of *Fungi Columbiani* and *North American Uredinales* entailed the identification, putting into packets, labeling, and indexing of 427,000 specimens.

On his collecting trips, which took him into every State in the Union, as well as into Canada and Mexico, Dr. Bartholomew personally collected 290,672 specimens which were later identified and appropriately cared for. On his journeys he discovered approximately 470 species of fungi that were new to science. In the work of identifying specimens and the naming of new species he was always closely in touch, either in person or by correspondence, with such men as J. B. Ellis, Charles H. Peck, E. W. D. Holway, J. C. Arthur, Ellsworth Bethel, John Dearnness, and many others. He had the pleasure and the inspiration of making collecting trips with all of the men whose names have been mentioned, and with a host of others.

Dr. Bartholomew was always in close touch with much of the botanical work done by the United States Department of Agriculture. From 1891 to 1893 he was engaged by W. T. Swingle, of the Department of Agriculture, to conduct on his farm, which had become the garden spot of that part of Kansas, a series of tests relative to the control or eradication of grain rusts by spraying or by soil treatments. Accounts of some of the results of this work may be found in the Department Year Book for 1892 and in the Journal of Mycology for 1893. Again, under the direction of the U. S. Department of Agriculture, from 1908 to 1913, Dr. Bartholomew conducted an extensive series of tests on the growing of various promising types and strains of corn, cotton, and alfalfa from many different parts of the world. Over 100 different species or types and strains of alfalfa were growing on his farm at one time.

Dr. Bartholomew had several invitations to become connected with colleges and universities, but he always refused until in 1929 when he became curator of the mycological herbarium at the Fort Hays Kansas State College. He gave as his reason for refusal that he preferred to work in private on the farm which he homesteaded in the waning days of the buffalo and antelope; the farm where he had planted hundreds of shade and fruit trees. Although he was reluctant to connect himself with one of the institutions of higher learning, he felt highly honored when in 1898 the Kansas State Agricultural College conferred upon him the honorary degree of Master of Science, and again in 1927 when the same institution conferred upon him the honorary degree of Doctor of Science.

Dr. Bartholomew published few scientific articles. Many might have been written concerning new species which he discovered, criticisms of species already named, host and parasite distribution, and the alternate hosts of certain parasitic fungi, all of which interested him greatly. His correspondence is rich in such things, but he left this work for others to do. In 1899 he published "The Plant Rusts of Kansas" in the Kansas Academy of Science. In 1927 he published "The Fungus Flora of Kansas" as Contribution No. 268 of the Kansas State Agricultural College. This publication lists 1,829 species as having been found in Kansas. Almost 20 per cent (360) of these were new to science. Up to the time of this report only about 465 species had been listed as having been found in Kansas. The great increase was due almost entirely to the work of Dr. Bartholomew. In recommending to the Dean that the manuscript for "The Fungus Flora of Kansas" be published, Dr. L. E. Melchers said, in part: "Dr. Bartholomew is the country's foremost collector of fungi and a student of mycology. . . . It is largely due to Dr. Bartholomew's research efforts that Kansas ranks so high in its report of so large a number of species of fungi." Fifty-eight additional species were reported for Kansas by Dr. Bartholomew in the "Trans. Kansas Acad. Sci." 33: 82-83. 1930. He personally collected all of these but three.

Dr. Bartholomew's most extensive work was published in book form in 1927 and was entitled "Handbook of the North American

Uredinales." This book included the names and synonyms of all of the plant rusts that had been found up to that time in North America, Greenland, and the West Indies. The list contains 1,240 species and 3,505 synonyms. A revised edition of this book was published in 1933.

Dr. Bartholomew was a member of the American Association for the Advancement of Science, Kansas Academy of Science, American Forestry Association, Mycological Society of America, American Phytopathological Society, and the Delta Upsilon honorary society.

The terms neat, methodical, and accurate characterize the work of Dr. Bartholomew. In collecting and putting up of specimens, "Good enough is not good enough," was an expression he often used. He did not measure success in terms of speed of accomplishment but in terms of reality and completeness. These traits, coupled with his seemingly inexhaustible supply of energy and zeal for accomplishment, perhaps explain how he could remain a successful farmer during the earlier years of his mycological career, and how, in spite of the fact that he was far removed from all of the leading educational and scientific centers, he became a world figure in the field of mycology. Another indication that he was a hard and methodical worker is shown by the fact that in spite of his many other arduous duties, he kept a well-written diary for 19,000 consecutive days (52 years), and it was from this diary that most of the information in this article was taken. A life sketch of Dr. Bartholomew would not be complete without stating that he was interested in and took part in civic and educational matters to the fullest extent of his ability. He would also wish to have a tribute paid to the one who so faithfully encouraged and assisted him in all types of work with which he was concerned, his wife, who survives him, Rachel Isabel Bartholomew.

# A NEW PUFFBALL

ELIZABETH EATON MORSE

(WITH PLATES 12-15)

The Gasteromycete described in this article appears to be the most abundant and widely distributed puffball at high altitudes in the western states. In the herbarium of the University of California specimens of this species had been confused with *Calvatia sculpta* (Hark.) Lloyd,<sup>1</sup> and we had no suspicion that they might be a different species until an inquiry accompanied by a specimen arrived from Doctor P. F. Shope (1933) concerning a Rocky Mountain puffball which has an excessively branched capillitium. Prompted by his inquiry, we examined all our material labeled *Calvatia sculpta*, and found that over one-half of it had the same peculiar capillitium as the Colorado plant. During nine years, the writer has collected in many different localities many examples exhibiting the peculiarity mentioned as well as other characters in common.

This puffball has a peridium which suggests *Calvatia caelata*, *C. sculpta*, *Scleroderma flavidum*, and *S. aurantium*; a deep, sterile, rooting base which suggests *Bovistella*, and a discrete, excessively branched thread which suggests *Bovista*. *Bovistella* and *Mycenastrum*. But it cannot be a *Calvatia* because of its much branched, entangled capillitium; it cannot be a *Scleroderma*,<sup>2</sup> because *Scleroderma* has a single layered peridium and a scanty, fragmented capillitium; it cannot be *Bovistella*, because it does not have an apical mouth; it cannot be *Bovista* because it has a sterile base, is not a "tumbler," and has no apical mouth; it cannot be

<sup>1</sup> Lloyd, Myc. Writ. 1: 203. 1904; Setchell, Sierra Cl. Bull. 6: 39. 1906; Setchell, Bull. Torrey Club 35: 291. 1908; Morse, Sierra Cl. Bull. 14: 61. 1929; Morse, Nat. Mag. June 1931.

<sup>2</sup> We find in Sacc. Syll. 7: 140. 1888, *Scleroderma fragile* (Lév.) De Toni (*Mycenastrum fragile* Lév.); *S. fragile* has a "nude" peridium and no columella. Patouillard has *Mycenastrum martinicense* with peridium broken up like our plant, but it has a central columella. Our puffball does not fit any of these descriptions. Bull. Soc. Myc. Fr. 18: 178. 1902.

*Mycenastrum*, because it does not dehisce in a stellate manner, and it is deeply rooted in soil. Externally, it closely resembles *C. sculpta*—in fact it has always been supposed to be *C. sculpta*, as previously stated, but is very distinct in the character of the capillitium and of the spores. Structurally, it has some characters of both *Bovistella* and *Mycenastrum*. In other words, we have here a puffball which bridges the gap between two large, distinct groups of puffballs: it has characters which, on the one hand, look to the *Lycoperdon* and *Calvatia* group, and, on the other hand, to the *Bovista*, *Bovistella* and *Mycenastrum* group.

With respect to the correct classification of this puffball we have conferred with mycologists on both sides of the Atlantic and have received several different opinions. We are concurring with the view of Professor W. C. Coker when he says: ". . . from the evidence before me I cannot find that your puffball has ever been described. It seems to me that it is somewhere between *Calvatia* and *Bovistella*, and in reality does not belong to any genus as at present defined. I agree with you that it is not a *Mycenastrum*."

A rich collection was recently made at Soda Springs, California, from which ample material was secured for completing studies of the early stages of development.

#### **Calbovista gen. nov.**

Sporophore medium to large, cremaceous, top-shaped, solid base ending in soil-embedded rhizomorphs. Peridium two-layered: the outer thick, coriaceous and broken into pyramidal plates which fall away from top downward at maturity; the inner layer a delicate membrane. Gleba fragile, dark umber at maturity; subgleba present, distinct, deep. Capillitium abundant, discrete, ochraceous yellow, antler-like. Basidia four-spored.

#### **Calbovista subsculpta sp. nov.**

Sporophore cremaceous, irregularly top-shaped, averaging 8 (16) cm. wide by 9 cm. deep, often plicate and contracted towards the rooting base; peridium<sup>3</sup> two-layered, the outer thick, cori-

<sup>3</sup> The great variation in size of mature plants of both *Calvatia sculpta* and *Calbovista subsculpta* may be accounted for by their varied habitats. It should be borne in mind that these puffballs often grow where the snowfall is exceedingly deep, and consequently when the melting season arrives the supply of moisture is continuous for several weeks. Other conditions re-

aceous, broken into irregular three to six sided, low pyramids—usually blunt, sometimes pointed; pyramids 5–8 mm. thick on top of sporophore, gradually becoming shorter on sides, peridium quite thin towards the base. Pyramids show parallel markings<sup>4</sup> on their sides, similar to those found in *Calvatia sculpta*. Inner peridium, an extremely thin, shiny tissue, depressed into areas by the heavy pyramidal plates; sterile base, one-fourth to one-third of the gleba consisting of chambers of moderate size, persisting after fertile tissues are dispersed and becoming more or less purplish with weathering; diaphragm absent; fertile tissues, in young plants not readily distinguished from sterile, pass through color changes when maturing—white to sulphur yellow, to golden brown or mummy brown (Ridgway), to dark umber, always darker than *C. sculpta*; deliquescence of gleba free but never complete.

*Microscopic characters.*—Glebal chambers closely adjacent, lined with clavate basidia, 10–12.5  $\mu$  long by 5–7.5  $\mu$  wide, each basidium bearing four spores; sterigmata usually short, 1.7–5.5  $\mu$ , the longest set 7.5  $\mu$ , length uniform on each basidium. Spores globose, 3–5  $\mu$ , ochraceous brown, smooth to faintly warted, uniguttulate, with a hyaline pedicel up to 2.5  $\mu$  long; episporule a half micron thick. Capillitium free, consisting of short, discrete units with abundant antler-like branching, much entangled; secondary branches bluntly pointed, not varying much in width from main branch; threads 5–10  $\mu$  wide, wall thick up to 2.5  $\mu$ , becoming thinner towards the tips; not septate,<sup>5</sup> not pitted, ochraceous yellow, concolorous.

**HABITAT.** In disintegrated rock mixed with soil or in open coniferous forest, 3,000–11,000 feet above sea level; may be associated with *Calvatia sculpta*, but not exactly in the same colony; a colony of each species at Soda Springs about fifty yards apart.

**HABIT.** Gregarious, usually single, but occasionally caespitose.  
**SEASON.** Species collected April to August.

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maining favorable, both these species may continue to grow for many days; the largest weight recorded is of *C. sculpta*—four and one-half pounds, General Grant National Park (Roberts). The spiny covering of young plants is often disproportionately thick.

\* These markings are probably attributable to the variation in temperature of night and day (up to 40° F.); plants do grow during the cold nights, but growth is slower than in the warm daytime and the change of rate leaves a record on the sides of the pyramids.

<sup>5</sup> Threads of *Bovista* and *Bovistella* also not septate in the species examined by Cunningham. Proc. Linn. Soc. of N. S. W. 50: 368. 1925.

*Type collection and locality.* Description composite, based on a large collection taken by the author at Soda Springs, California, elevation 6,767 feet, May 7–May 23, 1934. Plants studied and regarded as typical are deposited in the herbarium of the University of California as no. 525436.

**DISTRIBUTION.** Colorado: Boulder (Shope).  
Idaho: Moscow (Diettert).  
Washington: Mount Rainier, Paradise and eastern area (Brockman, Diettert).

California: Mount Shasta City (Whiteley, Morse); Mount Shasta, below Medicine Lake (Morse); Drakesbad and Mineral, Mount Lassen National Park (Morse); Chester, Lassen region (Martin, Gay, Morse); Battle Creek Meadow (Jepson); Quincy, Plumas Co. (Burdick); Merrimac, Butte Co. (Norman); Cisco, Placer Co. (Gould, Cree); Soda Springs, near pass over the Sierras above Truckee and Donner Lake (Jones, Lemon, Saunders, Barnes, Morse); Alpine Lake, Pine Crest, Dardanelles (Morse); Calaveras Big Trees (Wirt); Stanislaus National Forest (Patty); Eagle Meadow, Tuolumne Co. (Grant, 1915); Yosemite National Park (Harwell, Morse); Huntington Lake (Pierson); Rock Creek, east side of Sierras, below Mono Pass, above Bishop (Matthews); General Grant National Park (Roberts, Cunningham, Morse); Sequoia National Park (Dixon, Bracelin, Forster, Morse); Big Pines Park, Los Angeles Co., 7,000 feet elevation (Templeton); Bluff Lake, San Bernardino Co. (Nicholson, 1920).

The genus name proposed suggests the genus *Calvatia* to which it is closely allied and, incidentally, California, where the largest collections have been made; "bovista" suggests both *Bovista* and *Bovistella*, which also have discrete, branched capillitrial threads. The species name suggests *C. sculpta*, whose peridium it simulates, and for which it has been mistaken during many years. Discussion and criticism of the opinions and statements herein presented will be welcomed.

I wish to make grateful acknowledgment to Doctor P. F. Shope, University of Colorado; Doctor Carleton Rea, England; Doctor John Dearness, Canada; Doctor W. H. Long, New Mexico; Doctor C. L. Shear, Washington, D. C.; Doctor W. C. Coker and Miss Alma Holland, University of North Carolina; Doctor Lee

Bonar, University of California; Miss Vera P. Mentzer, for assistance in the microscopic studies; also, the numerous and interested collectors of abundant material.

CALIFORNIA MYCOLOGICAL SOCIETY,  
UNIVERSITY OF CALIFORNIA,  
BERKELEY, October 1, 1934

#### EXPLANATION OF PLATES

Photographs and Photomicrographs by W. C. Matthews

##### PLATE 12

*Calbovista subsculpta*: Left, Section of young sporophore; gleba white; rhizomorph solid. Yosemite; Center, Sporophore globose 9 cm.; peridium broken into plates with appressed pyramids, having parallel side markings. Below Yosemite Falls; collected by the author; Right, Peridial plates small, pyramids elevated, tips connivent. Moscow, Idaho; collected by Diettert.

##### PLATE 13

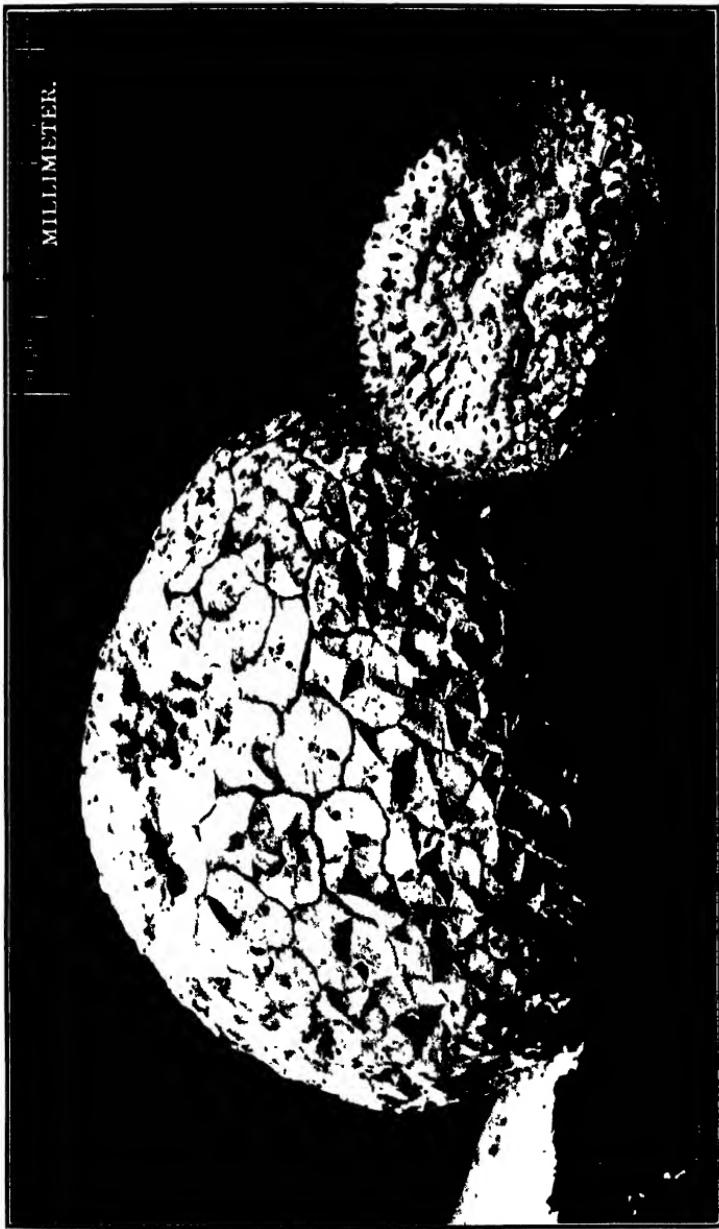
Figs. 1-6 inc. *Calbovista subsculpta*: 1, Sporophore large, flattened globose; gleba depressed into shallow discs by heavy plates, the discs lined by delicate endoperidium which soon disintegrates and merges with glebal tissues. Solid, anastomosed rhizomorphs of hyphae and agglutinated sand. (Grew on the bank of a creek, and when spread out were large enough to fill an ordinary cigar box.) Sequoia National Park; collected by Forster; 2, Young sporophores, perfectly white; peridia cracked. Idaho; collected by Diettert; 3, Vertical, median section; deep sterile subgleba; gleba white; 4, Young stage; peridium cracked. Camp Curry, Yosemite; collected by the author, 1926; 5, 6, Caespitose. Soda Springs, near summit of Sierra; collected by Saunders and Lemon, 1934.

##### PLATE 14

Figs. 1-5 inc. *Calbovista subsculpta*: 1, Peridial plates large, deep, heavy. Soda Springs; collected by Saunders; 2. Sterile base, dark umber, tinged purplish, embedded in soil; persists a long time. Exoperidium cracking off; endoperidium intact. Yosemite; collected by author, 1926; 3, Section from a Boulder, Colorado plant; peridium and glebal tissues characteristic; collected by Shope; 4, Subgleba deep; gleba beginning to turn dark. Sequoia National Park; collected by Dixon; 5, Reverse of fig. 4, pl. 13.

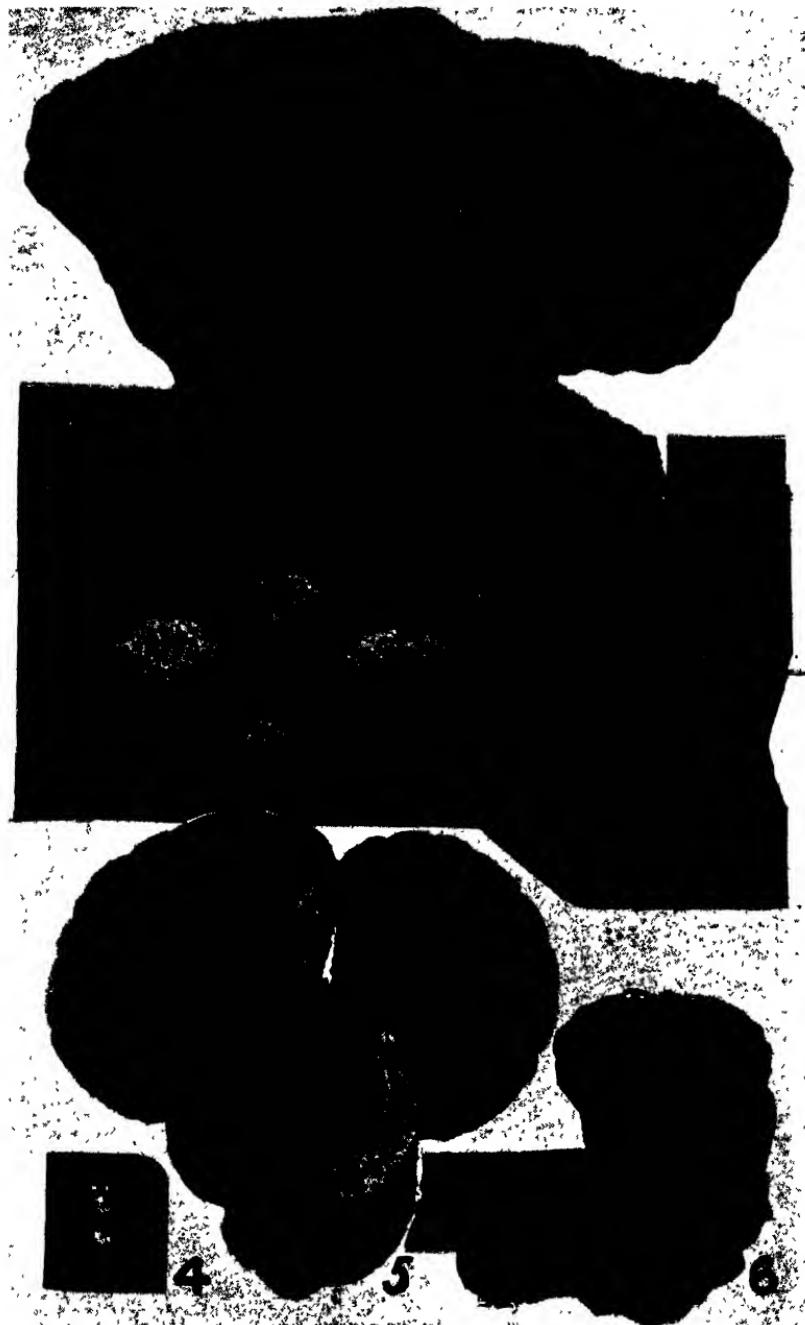
##### PLATE 15

Photomicrographs of capillitrial threads and spores. X 383. A, *Mycenastrum Corium*; B, *Calbovista subsculpta*; C, *Bovistella radicata*; D, *Calvatia sculpta*.



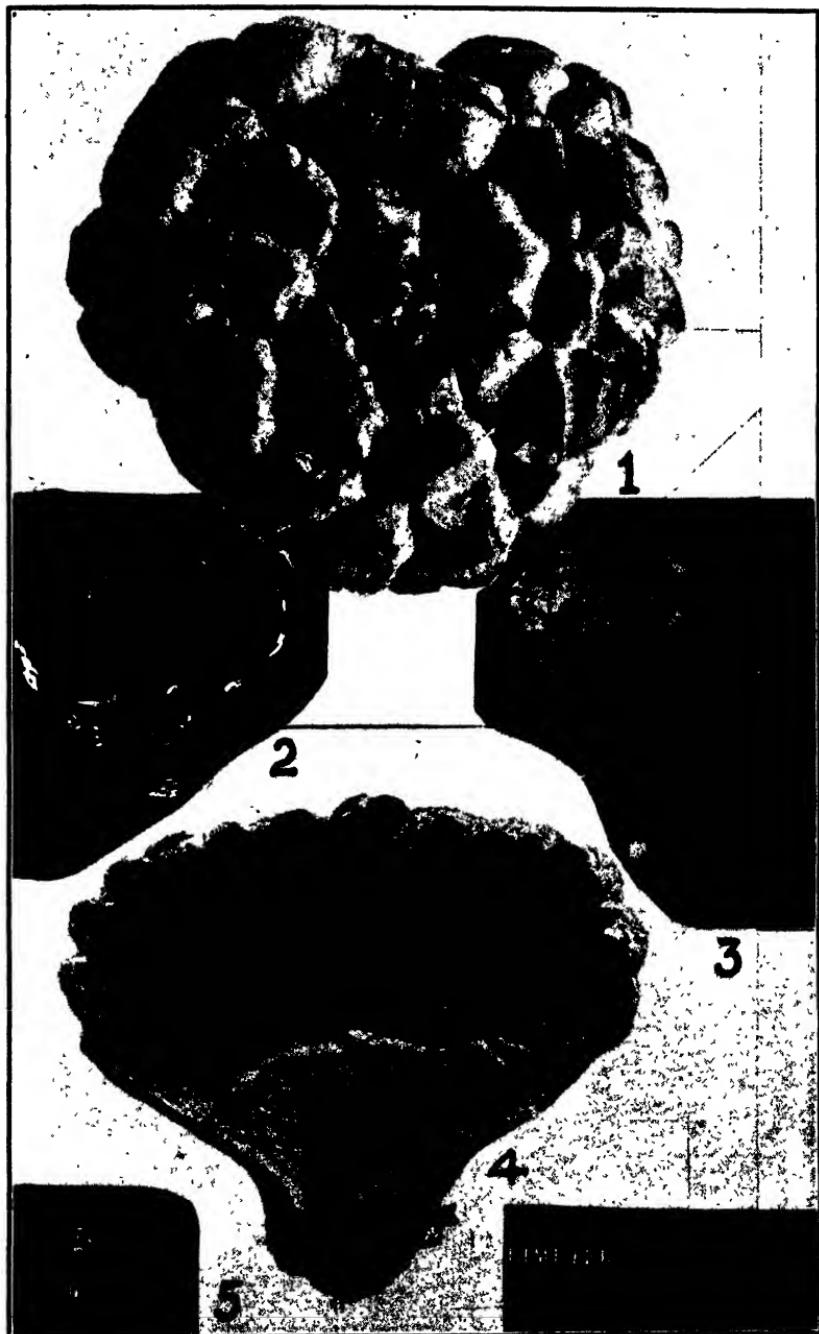
CALEIROVISTA SUBSCULPTA





CALBOVISTA SUBSCULPTA





CALBOVISTA SUBSCULPTA





A, *MYCENASTRUM CORIUM*. B, *CALBOVISTA SUBSCULPTA*.  
C, *BOVISTELLA RADICATA*. D, *CALVATIA SCULPTA*.



**Compared:**

1. All threads in A, B, C, D alike in one character, being tubular.
2. Threads in A, B, C discrete, i.e. entirely free from peridia. In D long, unbranched or rarely branched, growing from sterile base and endoperidium.
3. Branches in A short, stubby spines, limited to tips.
4. Branches in B antler-like, not much narrower than main trunks, abundant, much entangled.
5. Branches in C numerous on main trunks, elongated to slender, needle-like tips.
6. Spores in B, C, D small, fairly smooth, pedicellate. Spores in A large, very rough.

The capillitrial thread in Gasteromycetes is of great taxonomic importance. The thread in the new puffball is very distinct from that in each of the other three species shown in this plate.

## STUDIES ON ASCOIDEA RUBESCENS—II CYTOLOGICAL OBSERVATIONS<sup>1</sup>

LEVA B. WALKER

(WITH 78 TEXT FIGURES)

That *Ascoidea* shows characteristics of both Phycomycetes and Ascomycetes was pointed out by earlier workers, as Brefeld (2), Popa (11), and Holtermann (7). How strikingly this is true is attested by the fact that Lohwag, 1926 (8), concluded that *Ascoidea* is in every respect a Phycomycete while Varitchak, 1928 (12) and 1931 (13), considered it a simple Ascomycete. The fact that these two recent workers have arrived at such opposite conclusions makes the publication of these observations, largely completed before the publication of Varitchak's detailed paper, seem worth while.

Interest has naturally centered around the development of the spore sac<sup>2</sup> but the nuclear behavior in all stages of development has been studied.

### MATERIALS<sup>3</sup>

These studies are based upon materials collected at Ithaca, N. Y., in 1927 and at Lincoln, Nebr., in June, 1930, 1931, 1932, and 1933, and materials grown by the writer as previously described (14). Of the fixing fluids used, a dilute formal-acetic-alcohol (neutral formalin, 6 cc.; acetic acid, 1 cc.; and 50 per cent alcohol,

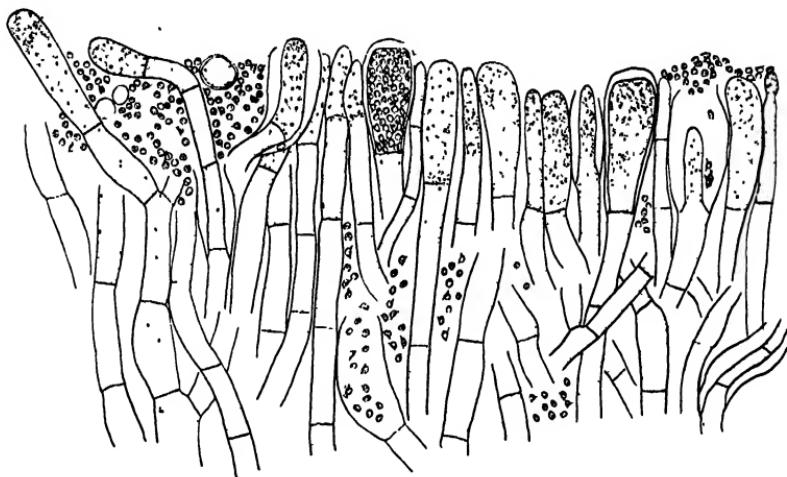
<sup>1</sup> Contribution from the Department of Botany, University of Nebraska, no. 90. I. History and Development. *Mycologia* 23: 51-76, fig. 1-74. 1931.

<sup>2</sup> In this paper the terms spore sac and spore are substituted for sporangium and sporangiospore used by Brefeld and by the writer in an earlier paper.

<sup>3</sup> In spite of the fact that *Ascoidea* is not well known in this country it seems to be widely distributed and very abundant when moist, warm, but not hot, weather prevails. Around Lincoln, Nebraska, it is commonly present on elms, both in woods and along streets, but disappears entirely after a day with temperatures above 95° F. Besides the first collection at Ithaca, N. Y., and numerous Lincoln collections, materials have also been sent the writer from Iowa City, Iowa, where it grew in a drain pipe, and from Greeley, Colorado, where it occurred on both cottonwood and elm.

93 cc.), Allen's modified Bouin's fluid, and Fleming's strong solution, have given the best results (in the order named), while the various mixtures of chrom-acetic have given the poorest. So much mineral matter is included in the fungus mass that pieces of the fungus placed in an acid fixing solution foam as would a piece of lime placed in the solution. For this reason, in later collections, the fixing solutions were repeatedly changed till foaming ceased before leaving for fixation.

The materials collected at Lincoln, because of the much greater abundance of spore sacs and their more definite orientation (FIG. 1), were much better for cytological studies. Fully as many spore



1.

FIG. 1. A cross section through the peripheral region of a young thallus of *Ascoidea rubescens* to show how reproductive and vegetative cells form a vague hymenium-like layer covering the surface of the thallus. Drawn by aid of camera lucida but telescoped by the omission of hyphal tips in two places.

sacs could be seen on a single slide made from many local collections as would be found on a hundred slides from materials collected at Ithaca. Continued observations on the fungus make it obvious that the collections from Ithaca were old, much depleted, and broken down by the myriads of organisms included in the mass. Materials grown in cultures contained so many depauperate and degenerating spore sacs that little use could be made of them ex-

cept for comparison. So far as could be determined, development was identical in all materials studied.

Serial sections, cut 3-7  $\mu$  in thickness, have been used. Sectioning has been very difficult because of hard granules included in the fungus mass. For this reason most of the series were more or less torn and broken. The most satisfactory stains employed were Heidenhain's iron-alum haematoxylin and Fleming's triple stain. The haematoxylin followed by orange G in clove oil gave especially clear differentiation. For staining toto mounts of germinating spores, Brazilin was much better than other stains used. Toto mounts never gave clear differentiation because of the large size of hyphae, spore sacs, and conidia, and the extremely small size of the nuclei. The only stain that gave clear differentiation of the nuclei in toto mounts was secured by using Barrett's smear technique (1 pt. equal pts. of stock iron-alum and haematoxylin, 1 pt. 95 per cent alcohol, 2 pts. glacial acetic acid) but so many crystals usually formed among the hyphae that details were obscured. Also the walls of mature endogenous spores were impermeable to the fluid. With other stains employed it was impossible to secure clear differentiation through unbroken walls.

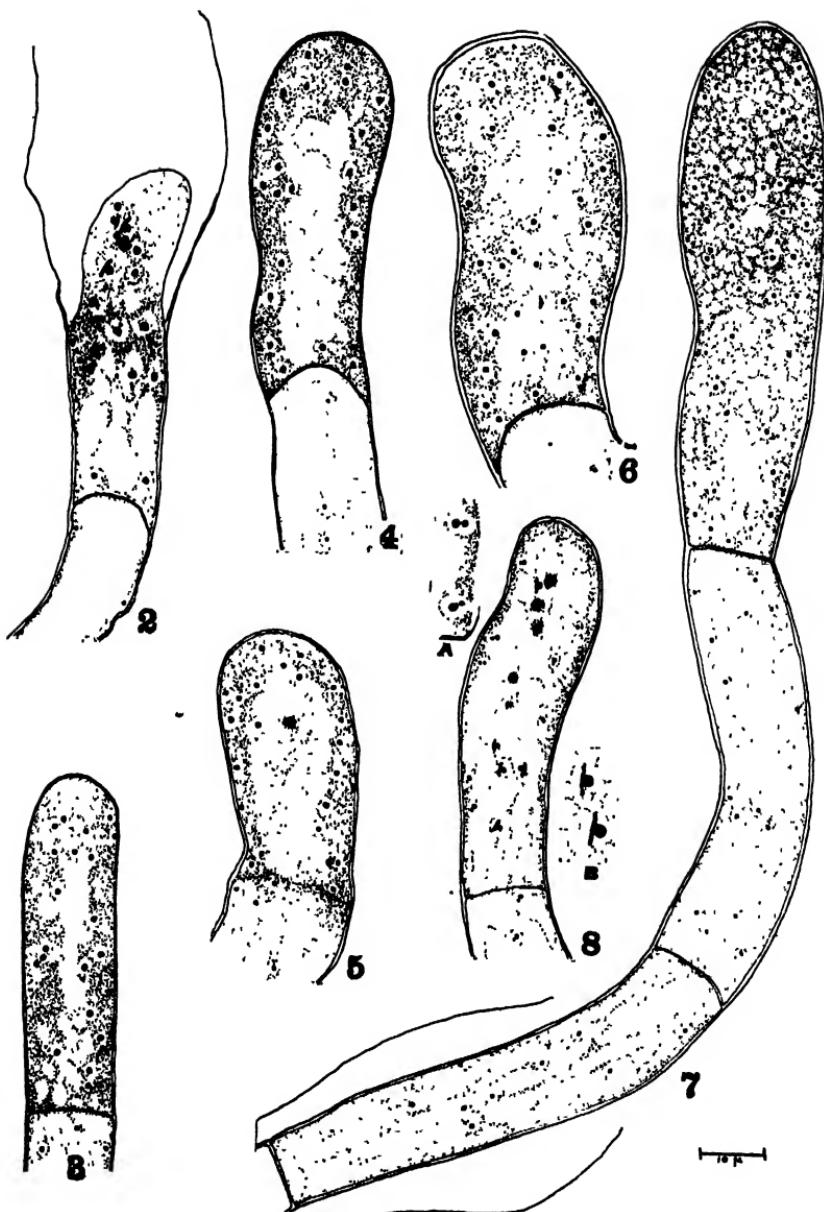
#### CELL CONTENT

All cells formed by *Ascoidea*, except the spores of the spore sac, are coenocytic. Each nucleus typically contains a relatively large nucleolus and little additional chromatin. The nucleus and especially the nucleolus, are variable in size, being much larger in young cells than in older cells (FIG. 2-15), but structurally they are similar. Many nuclei in hyphae that show other indications of degeneration contain irregular, granular, chromatic masses, such as are shown in spores (FIG. 38, left). Similar nuclei are also occasionally found in vigorously growing hyphae.

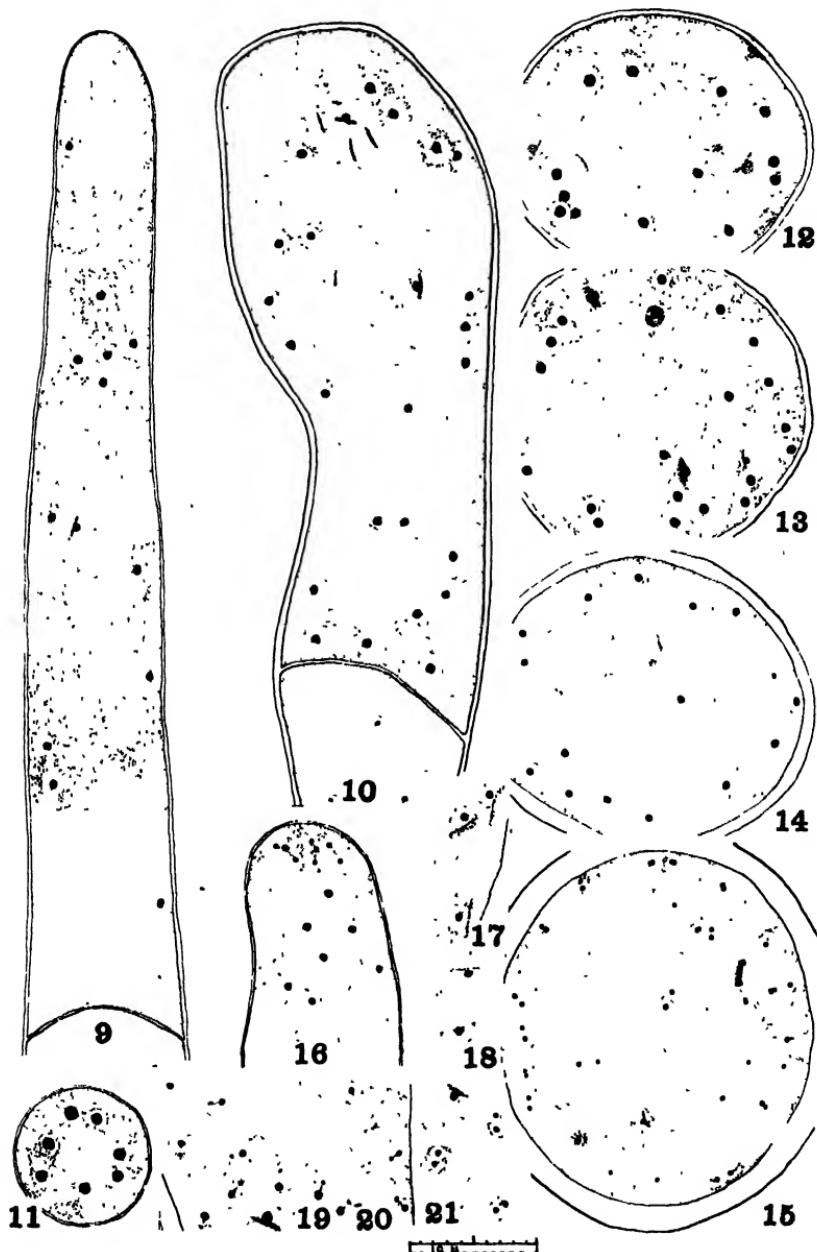
Mingled with the nuclei, especially toward the apex of young cells but occurring in all coenocytic cells, are extremely conspicuous, deeply staining bodies of several types. These are most conspicuous in materials fixed with fluids containing osmic acid and stained with haematoxylin but are evident with all fixing fluids and stains. The most conspicuous and definite of these bodies, in surface view, look like densely stained nuclei with extremely large

nucleoli (FIG. 8 AND 13). In side view, however, they appear as deeply stained rods with a homogeneous deeply stained globule attached laterally at the center. Through varying views it is obvious that these bodies consist of a circular disk of granular matter and a globule of homogeneous, highly refractive consistency. These "disk and globule" bodies are usually about the size of the nuclei in the cell, or larger, but in the same cell some may be two or three times as large as others (FIG. 2, 5, 8, 10, 12-15, 19-22, etc.). The origin and identity of these bodies are very perplexing. They are probably degenerating nuclei since what seem to be intermediate stages have been observed. It is possible that nuclei such as shown in figures 17 and 18 represent beginnings of this degeneration. Later stages appear as disks and globules but not so dense. They are most abundantly present in young vigorously growing tips of hyphae developing on old, much elongated hyphae and are much less abundant in tips of young hyphae that have not yet formed many cells. In coenocytic hyphae such as these degenerating nuclei might easily be carried forward in the cytoplasm and this may readily account for their occurrence as observed. Much oil is stored by the fungus and the possibility that they may be structures associated with oil secretion has suggested itself. These bodies are so highly refractive that they can be seen in living hyphae, especially in side view, where the disk appears as a rod-like body. These are undoubtedly the structures that Varitchak (13) considers meristematic or sex nuclei. That this is not the case is shown by the facts (1) that they are rarely found surrounded by dense cytoplasm and are usually found in much vacuolate parts of the cell and (2) that they are present at all stages of development except in endogenously developed spores, even occasionally in the slime surrounding developing (FIG. 35 AND 75) and mature spores. The fact that following some fixing solutions they do not stain at all as do nuclei is also a strong reason for considering that they are not nuclei.

Bodies which appear like disks and globules separated from each other also abound, especially in older parts (FIG. 10 AND 35). Various sorts of deeply staining bodies that have a fibrous to crystalline appearance are commonly seen. These are especially



Figs. 2-8. Details of hyphal tips and young spore sacs to show form and protoplasmic content. 2, a proliferating hyphal tip; 3, a young vegetative tip; 4-6, young spore sacs; 7, an older spore sac on a proliferating hypha; 8, degenerating spore sac.



Figs. 9-15. Nuclear variations. Longitudinal section of a young spore sac, 10, and vegetative cells, 9 and 16, to show similarity of nuclei; 11-15, cross sections of a hyphal tip (11) and spore sacs (12-15) in various stages of development up to the beginning of spore formation to show decrease in nuclear size as related to the thickening of the wall of the spore sac; 17-21, types of nuclei commonly observed, especially in cells with scanty cytoplasm.

frequent during the earlier stages of endogenous spore development (FIG. 15, 35, AND 36) but may occur in other cells.

#### MYCELIUM

Except for the hyphal tips, the mycelium is composed of cells with scanty protoplasmic contents. The ultimate cells, on vigorously growing hyphae, are filled with deeply staining protoplasm usually containing from 6 to 20 nuclei. When an apical cell is very young the protoplasm is of quite uniform peripheral density with many small central vacuoles (FIG. 3). As the hyphal tip elongates the protoplasm is pushed forward and a large vacuole is formed near the base of the cell (FIG. 9). When this basal vacuole has enlarged to about two-thirds of the length of the cell (FIG. 1, left of central mature sporangium) a cross wall is formed just below the dense protoplasm in the upper third of the cell. In the penultimate cell the protoplasm is much less dense and is largely peripheral. The protoplasm becomes successively more and more scanty till in old hyphae all active protoplasm seems to have disappeared. Older hyphal cells with dense protoplasmic contents are occasionally found and it is from these that branches develop. Such cells occur often even in old, much broken-down materials and are capable of initiating new growth. In general, hyphal tips destined to continue vegetative growth, maintain approximately the diameter of the hyphae from which they arise (FIG. 3), those preparing to form conidia become narrowed at the tip (FIG. 9), while those about to form spore sacs widen apically (FIG. 4-7). The protoplasmic contents are similar in all. The spore sacs usually develop on hyphae of somewhat larger diameter than those forming only conidia.

Because of apical growth the fungus when growing vigorously forms smooth, more or less cushion-like masses. In these masses, which may be over a centimeter thick, the center is composed of seemingly empty hyphal cells and all development is confined to the peripheral region. Thus in a cross section through one of these masses the surface layer gives much the appearance of a hymenium. The interhyphal spaces are filled with watery ooze which envelops the hyphae to their tips. In stained sections its extent is clearly visible because of granules at the surface. All reproductive struc-

tures develop near the surface within this saturated region. Figure 1 is a somewhat diagrammatic representation of these characteristics as seen in a single section of material abundantly developing spore sacs. In younger material the surface would be occupied by conidial structures.

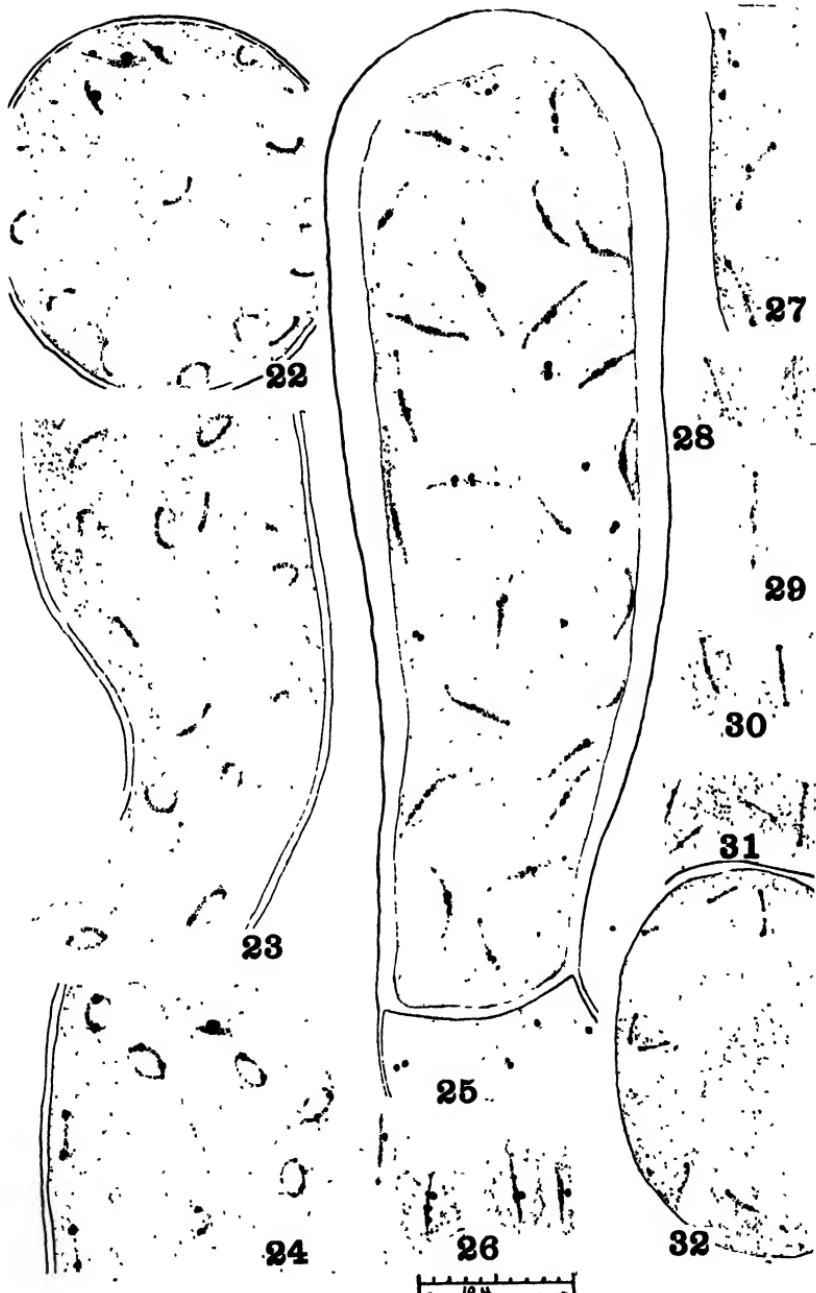
#### DEVELOPMENT OF SPORE SACS

Typically, spore sacs develop on hyphae which for several cells back are broader at their apical than their basal ends (FIG. 1, many cases, and 7) but many exceptions to this were found (FIG. 1, spore sac at extreme left). In all cases the ultimate cell itself becomes apically enlarged as the earliest definite indication of its differentiation (FIG. 4 AND 5). In some cases at least, the differentiation is evident even before the cross wall below is developed (FIG. 5). The nuclei in all tips, whether about to give rise to spore sacs, conidia, or vegetative growth, are so similar that they are indistinguishable (FIG. 3, 5, 9, AND 10). As a spore sac becomes larger and older an enormous multiplication of nuclei occurs, the walls thicken, and the centrally vacuolate condition is replaced by denser protoplasm (FIG. 4-7, 10, AND 12-15). The nuclei in older spore sacs look like those in older vegetative cells but are surrounded by dense rather than scanty cytoplasm.

Spore sacs vary so greatly in size and nuclear content that it is very difficult to estimate the age of a given spore sac during its earlier stages of development. The thickness of the wall as correlated to nuclear size seemed the best criterion available but it was not entirely dependable. In older spore sacs the wall is much thicker toward the apex than at the base. A series of cross sections beginning with figure 11, a cross section of a hyphal tip, and followed by figures 12-15, show successive changes in the nuclear size and wall thickness up to the beginning of spore formation. So far as could be determined no multiplication of nuclei occurs after the stage shown in figure 15. Along with the multiplication of nuclei in the spore sacs there is very definite evidence of the degeneration of nuclei. In some spore sacs, developed under seemingly optimum conditions, few or no degenerating nuclei are found, while in others, where apparently growth is not so vigorous, they abound. In general, degenerating nuclei show granular,

deeply staining, chromatic masses instead of definite rounded nucleoli. They are considered degenerate because they are found constantly in cells whose cytoplasm shows the coarse granular condition characteristic of dead or dying cells.

Wherever nuclear divisions, which will be discussed later, were observed in spore sacs, all nuclei were dividing simultaneously (FIG. 22-32). Because of this an attempt was made to determine how many nuclear divisions occurred in a developing spore sac. The great variations in nuclear number in spore sacs made the results unsatisfactory. In counting on any one slide and averaging the number of nuclei in broader hyphal tips, young spore sacs with large nuclei, and spore sacs with spores, the numbers indicated three divisions in each case, as 20 for hyphal tips, 40 for young spore sacs, and 160 for mature spore sacs. This would indicate the existence of a stage with 80 nuclei between the 40 and 160-nucleate stages. Accurate counts on spore sacs with small nuclei were never possible because of their minute size and the presence of other chromatic materials in the cell for the counts made would make the estimated number (80) in the series mentioned seem possible. Whether hyphal tips such as these are ever transformed into spore sacs is questionable though possible. At least in many cases apical cells about to form spore sacs are clearly differentiated before the cross wall below is formed. These counts would indicate a series of 20, 40 (80), and 160 nuclei in a sporangium. Thus 3 or 2 divisions occur owing to whether counts on hyphal tips were or were not included. In order to secure more accurate counts on the number of spores in spore sacs than could be secured from serial sections, in the spring of 1933, isolated living spore sacs were mounted in thin films of water and crushed by pressure on the cover glass until the spores, held together by the intersporal slime, were separated so that they were only one deep in the mass. In making such counts from the same materials on two successive days the average number of spores one day was twice as great as for the other. (Thus if materials had been fixed the first day, and counts made, the conclusions would have been entirely erroneous. If counts could have been continued some ratio might have been established but a single hot day killed all materials both outside and in the laboratory.) The spores from



Figs. 22-32. Mitotic figures commonly observed, 22-24 before thickening of the wall of the spore sac, and 25-32 after thickening of the wall.

twenty-two living spore sacs were counted. The fewest number of spores found in this material was 42 and the largest 160. Counts of similar, large, well developed spore sacs in serial section gave as many as 159 spores. If the nuclei for a spore sac with 42 spores had arisen by 3 successive, simultaneous divisions the tip would originally have had 6 nuclei (6-12 (1 degenerate), 22 (1 degenerate), 42), and the one containing 160 spores would have developed from a tip with 20 nuclei (20-40-80-160) with no degenerate nuclei. Curiously this was the identical range that had been secured in counts of 36 typical hyphal tips from various materials. However hyphal tips do occur with fewer than 6 nuclei and more than 20. As reported in an earlier paper (14) one spore sac containing 8 spores was observed. If 3 divisions had occurred this must have developed from a tip containing a single nucleus! On the other hand, many especially broad hyphal tips, such as ordinarily give rise to spore sacs and were arbitrarily listed as young spore sacs, contain about 40 nuclei which may be the initial number. If so there could have been only two simultaneous divisions during the development of a spore sac. No definite cytological evidence was secured by which the point could be settled.

Even if it is impossible to say definitely whether two or three simultaneous divisions provide the nuclei for the spores developed in spore sacs, nevertheless counts show very positively that there is no multiplication of nuclei after the beginning of spore formation.

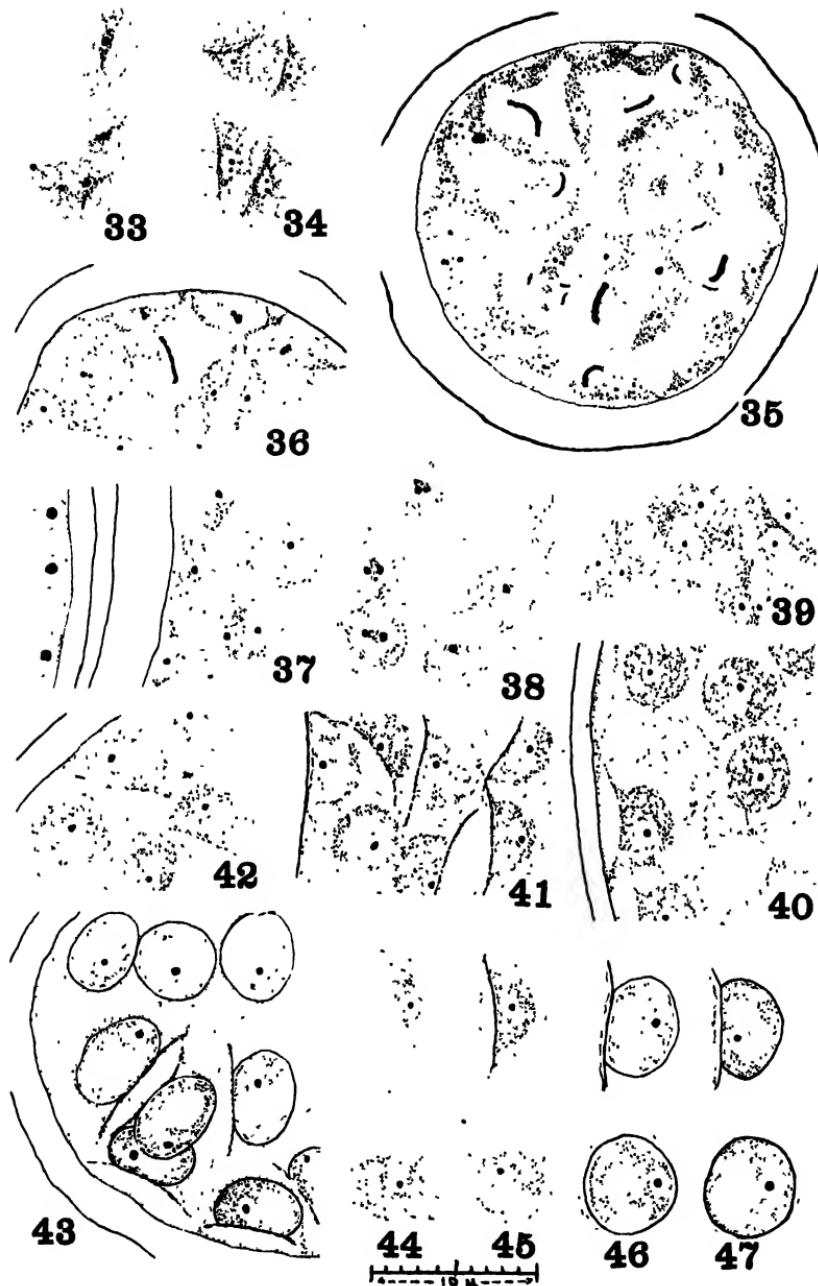
As a result of divisions in the spore sac the nuclei just preceding spore formation are the smallest observed—so small that it is almost impossible to observe details of behavior.

Figure 33 represents a possible beginning of spore formation and figure 34 shows a very definite, early stage in spore formation. The nucleus, which at all times shows indications of not being symmetrical in its organization, becomes surrounded by cytoplasm in a very unsymmetrical fashion. As a result of this, definite lens-shaped protoplasmic masses are formed which lie in thinner cytoplasm. The lens-shape mentioned is that of a circular lens, flattened or concave on one side and convex on the other. Thus when seen in surface view they are rounded and in side view more

or less crescent or spindle shaped. The flattened surface usually lies longitudinally in the sporangium and spores near the wall commonly, but not always, lie with the concave side toward the wall (FIG. 35-37). A clear area, probably a vacuole, is constantly located next to the concave side of the developing spore. The nucleus, during the early stages of spore formation, usually shows a nucleolus and a very small, deeply staining granule. The deeply staining granule may be entirely wanting. When present it may be located on any side of the nucleus as related to the shape of the spore. It has not been possible to attach any significance to its presence. As previously mentioned, many conspicuous, deeply staining, fibrous to crystalline, curved rod-shaped bodies of various sizes often abound in the thin cytoplasm between the spores (FIG. 35 AND 36) but are not universally present. The wall of the spore sac appears thicker at this time than at any other period during development.

The nuclei in these lens-shaped masses of protoplasm gradually increase in size and their surrounding cytoplasm becomes denser and thicker toward the center of the mass (FIG. 37-39). (In figure 37 a part of an adjacent young spore sac is shown for comparison of size of nuclei.) Figure 39 shows a few spores from near the center of a spore sac where they often lie closely appressed, flat surface to flat surface, or variously arranged side by side, seemingly as a matter of chance. In spore sacs that show other indications of degeneration as well as in vegetative cells and old spores the nuclei very commonly take on the appearance shown in figure 38.

In stages shown up to figure 40 the cytoplasm of the young spores is very homogeneous in structure. As the spores become more spheroidal many tiny vacuoles appear (FIG. 40) and each spore mass becomes surrounded on its convex surface by a transparent gelatinous-appearing layer. Soon the wall begins to form around the spore. It appears to form first on the concave side, but this is probably due to the fact that when looking at a side view one looks through the edge of a flattened surface because in surface view the wall there is no more obvious than on other parts of the spore. Curiously the wall over the flattened surface is deposited on the spore side of the clear area (vacuole) developed on



Figs. 33-47. Stages in the development of endogenous spores. 33-37, successive stages in the development of the spores; 38, young spores with degenerating nuclei; 44-47, a diagrammatic representation of spores as seen in side view (above) and surface view (below) in 4 selected stages of development.

the flat side of the spore, but over the rounded surface of the spore the wall begins to be deposited on the outside of the clear gelatinous-appearing area surrounding it (FIG. 41 AND 42). This clear area becomes narrower and narrower until the wall becomes closely adherent to the cytoplasm within the spore (FIG. 43). While the wall is being formed around the spore the vacuoles fuse to form the one large vacuole characteristic of the mature spores. Figures 44-47 show conventionalized spores during four selected stages of development. Side views are shown above and surface views below.

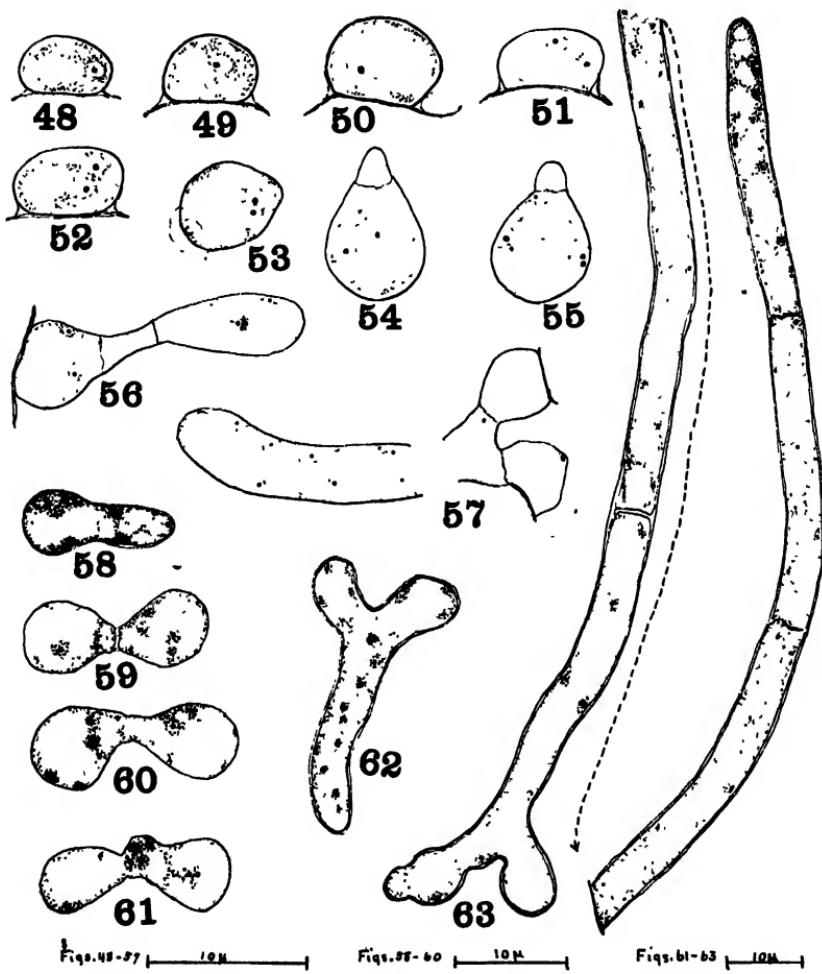
It seems evident that the "hat" shape of the spores results from the curious manner in which the spore walls are formed. The wall over the flattened surface extends to the outer area of the gelatinous appearing layer formed over the rounded surface. As the gelatinous layer becomes contracted as the spore matures the two walls at the edge of the flattened surface form the "brim" of the "hat." The development of the walls of the spores was best seen when stained with haematoxylin and counter stained with orange G in clove oil. The walls of spores stain like other mature walls but are much more impermeable as is shown by the fact that no stain would penetrate the spores when Barrett's smear technique was used.

The oily intersporal slime so conspicuous in fresh materials is soluble in the reagents used in the paraffin method so does not appear. Between the spores and lining the wall of the spore sac is a very delicate granular network that appears to be cytoplasmic (FIG. 35-43). Seemingly the slime is secreted in the meshes of this intersporal cytoplasm. This network becomes less and less definite as the spore sac matures and by the time of discharge is commonly entirely indistinguishable. At no time was a condition in any way resembling cleavage observed in a spore sac.

#### GERMINATION OF SPORES

In addition to toto mounts of germinating spores a few germinations were observed in sectioned materials. Figures 48-57 show successive stages as seen in sectioned materials, while figures 58-63 are from toto mounts. As was previously reported (14) germ tubes may arise from single spores or from the fusion of two

spores. Figure 48 shows a typical mature spore. As germination begins the spore swells, enlarging its vacuole (FIG. 49 AND 50) and the nucleus divides (FIG. 51 AND 52). Figures 53 and 54 show spores germinating while still binucleate, while figure 55



Figs. 48-63. Germination of spores. 48, a mature spore; 49-57, successive stages as seen in paraffin sections, and 58-63, as seen in toto mounts.

shows a similar germination stage with four nuclei. Figure 56 shows a spore with a germ tube with a single septum and its nuclear content. (The narrow isthmus in the germ tube is due to curvature and part being cut away.) It was almost impossible to

follow older germ tubes in sections. In figure 57, taken from two sections, the only definite case of a germ tube arising from the fusion of two spores, observed in serial sections, is shown. Figures 58-63, illustrating toto mounts, show the same behavior. Grossly these germination stages closely resemble similar stages in yeasts as shown by Guilliermond (4), but the nuclear behavior differs widely in that the spore nucleus in *Ascoidea* in all cases observed by the writer divides before germination and gives rise directly to coenocytic cells.<sup>4</sup>

#### CONIDIA AND THEIR GERMINATION

As has been previously stated conidia develop on hyphal tips which taper apically. From the tip of a hypha a slight enlargement develops which becomes filled with cytoplasm (FIG. 64). As this enlargement increases in size nuclei pass into it from the hypha below (FIG. 65 AND 66). As the conidium assumes its mature form a cross wall is formed to separate it from its bearer. The cross wall at first appears as a clear gelatinous layer at the base of the conidium in the midst of which a granular region of differentiation occurs (FIG. 67). Thus a new wall is formed on each side and the conidium separates readily from the cell below (FIG. 69). As has been described in an earlier paper, the conidia vary greatly in form. Thin-walled, cylindrical conidia such as shown in figure 70, are characteristic of young, vigorously growing hyphae and fall off very readily. Thicker walled, more globular conidia (FIG. 69 AND 71) are common on older hyphae, while especially thick walled conidia, such as shown in figure 72, are apt to form, as here shown, on proliferating tips following sporangial development and on or in other old hyphae. The nuclear content is similar in all cases. Each conidium contains many nuclei situated largely in the peripheral cytoplasm but the center is vacuolate.

Conidial germinations were observed only in toto mounts which were always unsatisfactory. Germinating conidia give rise to coenocytic germ tubes which may continue typical vegetative growth, give rise directly to conidia (FIG. 74), or, after the formation of a few cells, form small but typical spore sacs (FIG. 73 AND

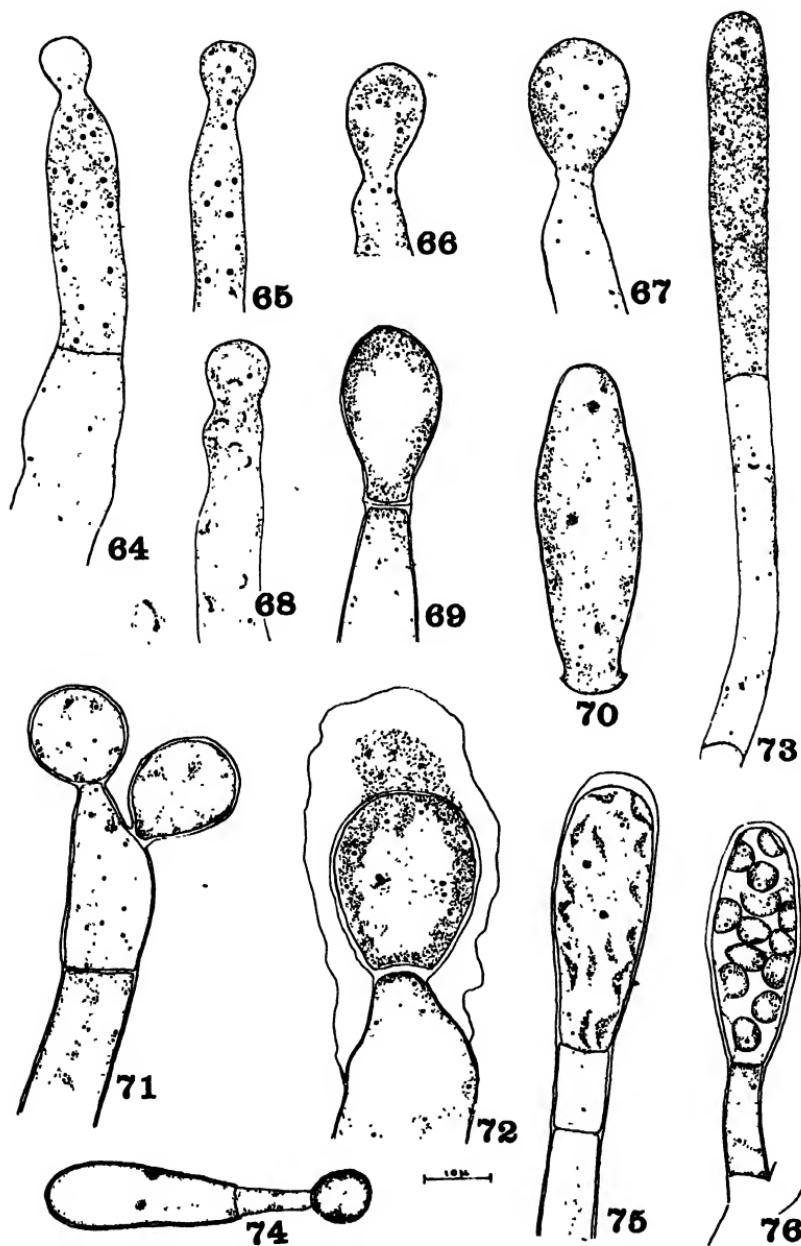
<sup>4</sup> Varitchak (13) figures some early germination stages that are unicellular.

75). Similar small spore sacs may develop from hyphal fragments (FIG. 76).

#### NUCLEAR DIVISIONS

Feeling that nuclear behavior, especially during the development of the spore sac, might not only be of interest but also helpful in determining the relationships of *Ascoidea*, more time was devoted to a study of this phase than any other. The results have been most disappointing. Chromatic conditions that at least resemble mitoses are commonly observed. In sporangia, where they are most commonly seen, the entire nongranular parts of the cell retain the chromatic stains so tenaciously that it is difficult to get a clear differentiation. Because of this and the fact that the stages observed do not show uniformity in chromatic behavior, the possibility that they are degenerative stages has been given much consideration. It seems probable, however, that they are mitotic stages because normal nuclei are never found in spore sacs showing such conditions. The granular cytoplasm in such cells seems perfectly normal. These stages have been considered mitotic by other workers on *Ascoidea* and will be discussed in this light.

Similar mitotic stages have been observed in spore sacs and occasionally in vegetative cells. In spore sacs the divisions appear to be simultaneous while in vegetative cells this is not usually true. In figures 22-32 an attempt has been made to show as accurately as possible the types of stages most commonly seen in spore sacs and the extent of the chromatic variations commonly seen in the same spore sac. These probably represent stages in the last mitosis preceding spore formation. Figures 22-24 show what appears to be a curious type of intranuclear spindle. In some cases (FIG. 22) the spindle seems rather short with granules at the poles while in others the spindle forms a nearly complete circle within the nucleus with very tiny granules opposite the heavier part of the spindle (FIG. 24, above and to right). Stages such as these were occasionally observed in vegetative cells (FIG. 68). When seen in spore sacs they always occur before the walls are greatly thickened. If the stages shown in figure 24 are properly interpreted, it seems probable that with the rupture of the nuclear membrane and the straightening of the spindle, stages such as are shown in figure 25 might result.



Figs. 64-76. Conidial formation and germination. 64-67 and 69, successive stages in conidial formation; 68, mitosis in such a hypha; 70, protoplasmic content of a long, thin-walled conidium, and 71 and 72, of globular thicker-walled conidia still attached to the cells which produced them; 74, a small conidium being formed at once from a germinating conidium; 73 and 75, small spore sac formed at the tips of short hyphae following germination; 76, a similar small spore sac formed on a short outgrowth from a hyphal fragment.

Stages similar to those in figure 25 are more commonly seen than any other stages. The spindle is always more or less curved. That these spindles are later in development than those shown in figure 24 is evident by the greatly thickened wall. It seems possible that the rupture of the nuclear membranes might release pressure upon the inner layer of the wall of the spore sac, which is gelatinous, and thus permit its sudden expansion. Four nuclei from a similar spore sac are shown in figure 26. Here it appears definitely as if the nucleolus was being ejected. Stages, somewhat similar to these, occurring in a hyphal tip, are shown in figure 16. Figures 27-33 show stages that undoubtedly develop later than those shown in figures 25 and 26.

Varitchak (13) as a result of his studies gives the sequence in nuclear division shown in figure 77, copied photographically from

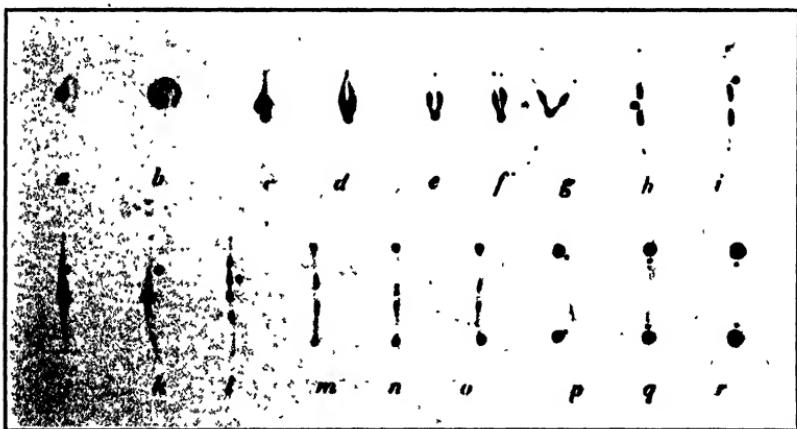


FIG. 77. Mitotic stages. A photographic reproduction of Varitchak's Fig. 2.

his figure 2. The nuclei shown in his figure 2, "a" and "b," are undoubtedly what the writer holds to be degenerating nuclei. For his figures "c-g," stages such as shown in figures 22-24 are possible. The writer would suggest stages such as are shown in figure 78 as a more probable series. Stages similar to all of Varitchak's (13) have been observed but those similar to his earlier figures appear to be degenerative stages. From his "h" on, his figures are characteristic except that when the chromosomes have reached the poles two definite chromatic bodies are often observed.

Nuclei such as shown in figures 8, above, 15, 19-21, 34, etc., have been a serious problem. It would seem that they might represent young nuclei that have just divided, such as might be expected to follow figures 30-32, and it may be that this is the case, as seen in figures 33-36. Very commonly, however, nuclei at this

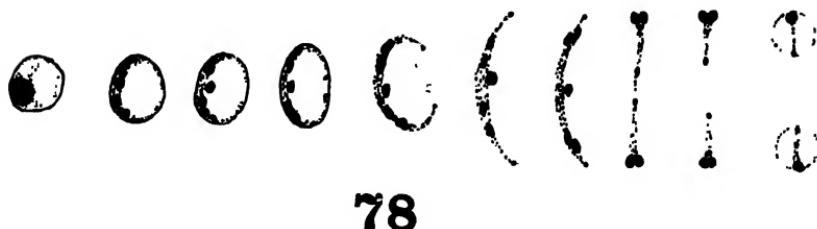


FIG. 78. Mitotic stages. A series of stages suggested as a result of these studies.

stage show only the one definite chromatic granule. Nuclei of this type are seen most commonly in old and depleted cells where the nuclei are small and active growth is not taking place. Their occurrence would indicate that they more probably represent either degenerative conditions or a type of amitosis in which figures 19-21 would constitute a series.

#### DISCUSSION

Forms such as *Ascoidea* that do not obviously fall within any of the usually recognized major groups of fungi are always of especial interest to students of fungous phylogeny. In this case the interest has centered around the spore sac and its endogenously developed spores. Three types of spore sacs are known among fungi: sporangia, asci, and gametangia. The spore sacs of *Ascoidea* were considered sporangia by Lohwag (8) who holds that *Ascoidea* is a true Phycomycete while Varitchak (13) concludes that *Ascoidea* is a primitive Ascomycete closely related to *Dipodascus albidus*. That the spore sac of *Ascoidea* was the result of the development of one gametangium in the absence of the other has been suggested by Atkinson (1).

From the description just given it is obvious that *Ascoidea* is not a typical Phycomycete. Its spore sacs resemble sporangia in that they are multinucleate from the beginning, develop without

nuclear fusions and at maturity contain a variable number of spores. The spore sacs of *Ascoidea* differ widely from sporangia in that the spores are not formed by progressive cleavage and at maturity are surrounded by cytoplasm in whose meshes an oily slime is secreted. While grossly this condition in the mature spore sac resembles the condition in certain Mucors where the intersporal spaces are filled by an oily slime, development is very different. Luhwag's studies (8) of *Ascoidea* did not include cytological observations on spore formation and this doubtless accounts for his conclusion that the spore sac is a sporangium and that *Ascoidea* is a true Phycomycete.

The spore sacs of *Ascoidea* are also surely very different from typical asci. No nuclear fusions were observed but simultaneous divisions occur in the spore sac. No astral rays were observed during spore delimitation but the spores are formed by a curious sort of free-cell formation and at maturity lie embedded in cytoplasm, similar to that surrounding ascospores in typical asci. Varitchak (13) has reported the fusion of two specialized nuclei in the young spore sac of *Ascoidea* and that this fusion nucleus by repeated divisions gives rise to the nuclei of the spores while all other nuclei degenerated. Hundreds of hyphal tips in all stages of development up to spore formation have been critically examined but no fusions of nuclei were ever observed. If the spores were formed by the repeated divisions of a fusion nucleus up to twenty (or forty) nuclei would regularly degenerate during the development of the larger spore sacs. Careful counts have shown that this was not the case. In old materials where developmental conditions were poor so large a percentage of degenerating nuclei might occur but in vigorously growing materials spore sacs abounded in which no indication of any degenerating nuclei were found. Thus indirectly, for the materials used in these studies, we have evidence against the probability of fusions and degenerations, as described by Varitchak (13).

The suggestion of Atkinson (1) that the spore sac of *Ascoidea* might represent an apogamously developed gametangium remains to be considered. In *Saprolegnia ferax* and related forms the oögonium regularly develops apogamous oöspores without the presence of antheridia. In such forms oöspores may even develop

in sacs looking like sporangia. In all cases in this genus the oöspheres arise by cleavage and there is no cytoplasm surrounding them nor the so-called oöspores which develop from them. In closely related forms the oösphere is surrounded by an abundant periplasm formed seemingly by the cytoplasm and degenerating supernumerary nuclei. In looking over the wide variations known to occur among Phycomycetes it would be easy to conceive of coenogametangia similar to the young spore sacs of *Ascoidea* in which many, instead of one, oösphere was differentiated and in which the oöspheres were surrounded by cytoplasm. The fact that two simultaneous mitotic divisions seem to precede spore formation in *Ascoidea* and in oögonia is at least an interesting coincident.

It has been commonly suggested by those who would derive the Ascomycetes from the Phycomycetes that the ascus may have arisen by the modification of the gametangium. In this connection, *Dipodascus* with its many-spored ascus arising from the fusion of a single nucleus from each coenocytic gametangium, has been taken as the simplest known form. Into a series such as this, *Ascoidea* as here described can easily be considered a related form in which the spore sac is a gametangium developed apogamously. The fact that no nuclear fusion was observed during the development of the spore sac does not in the mind of the author in any way detract from this relationship. The literature dealing with the subject has been so well discussed by Varitchak (13) that it will not be reviewed here.

It is surely such fungi as *Ascoidea* that form the links in a chain connecting Phycomycetes with Ascomycetes. Its phycomycetous relationships are obvious but it is even more definitely related to the lower Ascomycetes. Its two most outstanding ascomycetous characteristics are (1) its hyphae in which cross walls are not only present but formed so near the tip of the hyphae that the apical cells are usually, at the time of formation, the shortest cells in the hyphae, and (2) its spores are formed by free-cell formation. Harper's statement (6) that free-cell formation is perhaps "the most important and specific feature by which to distinguish the ascus from other spore-producing cells" is in general held by students of fungi. There are, however, no universally accepted criteria for the determination of border line forms such as *Ascoidea*.

Schröter (10) divided the Ascomycetes into three primary subdivisions. Hemiascomycetes, Protoascomycetes, and Euascomycetes. Into the first two groups he placed all forms lacking ascocarps. In a more recent classification Gäumann and Dodge (3) have in general included in the one group, Hemiascomycetes, the forms divided by Schröter into Hemi- and Protoascomycetes. In doing this the Hemiascomycetes are distinguished from Euascomycetes primarily by the presence of ascogenous hyphae in the latter group and their absence in the former. Two orders of Hemiascomycetes are recognized: the Endomycetales, which include those forms "in which the ascus arises directly as a product of the sexual act (wherever this takes place)," and the Taphrinales. It seems to the writer that *Ascoidea* definitely belongs in the Endomycetales as characterized above. Gäumann and Dodge (3) recognize three families in the Endomycetales, the Dipodascaceae, the Endomycetaceae, and the Saccharomycetaceae. The close relationship of *Ascoidea* to *Dipodascus albidus* of the Dipodascaceae has been previously pointed out. In the genera of the Endomycetaceae the hyphae are typically uninucleate, except when very young, but *Endomyces Magnusii* has coenocytic cells and even conidia. *Ascoidea*'s budding type of conidial formation and germination show characteristics common in species of the Endomycetaceae and Saccharomycetaceae. In these groups also there is a marked tendency towards apogamy, and in some known forms such as *Endomyces javanensis* and *E. capsularis* no nuclear fusions are known. If, in some strains, nuclei fuse in the young sporangium, as reported by Varitchak (12 and 13) this would only place it more definitely in line with the known variations in this order.

Another fact that connects *Ascoidea* definitely with the Endomycetales is the curious, hat-shaped spores which closely resemble those of *Eremascus fertilis*, *Endomyces deceptiens*, *E. fibuliger*, *E. Lindneri*, and *Willia anomala*. Not only do the mature spores resemble each other but Guilliermond (5) and Mangenot (9) also figure and describe young unwalled spore masses of similar form and a similar type of development. It seems probable that more detailed studies will show even greater similarities. One point of difference is that Guilliermond (5) always shows the flattened surface of the spore in some other position than toward the wall, as is

the most characteristic position for spores near the wall in the spore sacs of *Ascoidea*. Even the nuclei of these forms seem similar in all details so far as given. It is interesting to note that while free cell formation is described for all related forms, astral rays have not been observed. While of no especial significance, the fact that many of the well known members of the Endomycetales occur in slime fluxes or sugary solutions is an interesting coincident.

The confusion that has existed concerning *Ascoidea* seems only natural because it is neither a typical Ascomycete nor a typical Phycomycete. It seems to the writer, as also to Varitchak (13), that *Ascoidea* is closely related to both *Dipodascus* and *Endomyces* of the Endomycetales. The writer differs from Varitchak (13) in finding no fusions in the spore sac. Because no fusions were found, the writer considers the spore sac an apogamously developed ascus of a type intermediate between the asci of *Dipodascus* and *Endomyces*. The coenocytic characteristics of *Ascoidea* suggest a close relationship to *Dipodascus* of the Dipodascaceae, but spore formation is so like that in *Endomyces* that *Ascoidea* might as readily be placed in the Endomycetaceae as tentatively placed by Gäumann and Dodge (3). Because the ascus of all members of the Hemiascomycetes is so different from typical asci it seems fitting that, as suggested by Varitchak (13), and used by Gavaudan and Varitchak in recent publications, we should distinguish such asci by calling them *hemiasci* and the spores *hemiascospores*.

#### SUMMARY

1. For these studies materials collected at Ithaca, New York, in August, 1927, and at Lincoln, Nebraska, in June, 1930–1933, inclusive, were used.
2. The hyphae, conidia, and spore sacs are coenocytic but the spores formed in the spore sac are uninucleate.
3. The nuclei are much larger in young cells than older cells but all are similar in structure and are characterized by a large nucleolus and little additional chromatic material.
4. Characteristic chromatic bodies of several types are conspicuously present in coenocytic cells. Some of these at least arise from degenerated nuclei. The most conspicuous of these is a

granular disk to which a homogeneous highly refractive globule is centrally attached. These are evidently the bodies considered specialized nuclei by Varitchak.

5. Active growth is apical; new cells are formed as soon as the apical cell has elongated so as to be about a third longer than a typical cell. Cells below the apical region have scanty protoplasm and older cells lose their protoplasmic content.

6. All reproductive structures on actively growing thalli are apical, the surface of the thallus often appearing like a vague hymenium.

7. Hyphal tips contain up to 20 or more nuclei, young spore sacs up to 40 nuclei, and mature spore sacs up to 160 spores.

8. The nuclei for the spores formed in spore sacs arise by simultaneous divisions.

9. Spores are first differentiated in spore sacs as lens-shaped masses of denser protoplasm, flattened on one surface and rounded on the other, and are surrounded by less dense cytoplasm.

10. The characteristic hat-shape of the endogenously developed spores is due to the fact that the wall of the spore is formed next to the dense cytoplasm of the spore initial on its flat surface and on the outside of a clear layer of gelatinous appearance over the rounded surface. As the gelatinous layer disappears the "brim" of the "hat" is formed where these walls unite.

11. The spores formed in spore sacs enlarge and become two to several nucleate before forming germ tubes. They give rise to coenocytic hyphae either directly or after fusions.

12. Conidia of all types are multinucleate. They give rise either to conidia by budding or to coenocytic hyphae.

13. Nuclear divisions in the sporangium are probably mitotic, and probable stages are discussed. Similar figures were occasionally observed in vegetative cells. There are also some indications of amitosis.

14. The spore sac is probably an apogamously developed ascus.

15. These studies indicate that *Ascoidea* is a true Hemiascaceous fungus closely related to *Dipodascus* and *Endomyces*. The term hemiascus can well be applied to such asci.

The writer wishes to extend thanks to Prof. H. M. Fitzpatrick, of Cornell University, for his continued interest, and for sugges-

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# THE ASCOCARPS IN SPECIES OF PENICILLIUM

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(WITH 16 TEXT FIGURES)

Some form genera of the Ascomycetes have such definite conidial characters that their members appear to be of common origin. *Penicillium* may be placed in this category. Despite differences in type of conidial apparatus the species of *Penicillium* have many points in common so that this genus, considered wholly on the basis of the conidial stages of its species, properly circumscribed, has been believed to rest upon a somewhat different basis than many others. Over 400 species of *Penicillium* have been described on the basis of the conidial stages. The ascocarpic structures in the genus are relatively little known. The abundance of species of *Penicillium* and their economic importance justify further studies of this group. In view of these facts it is proposed to examine more critically some of the species which produce ascocarps. Such a study reveals certain extremely interesting features that appear during the development of the ascocarpic stage. Differences in the ascogonia and asci can be correlated with the group differences pointed out by Thom (1930); but these differences indicate lines of development more clearly than do the differences in conidiophores, as would be expected. Dr. Charles Thom has furnished several of the strains studied, and the author is also indebted to him and to Dr. B. O. Dodge who suggested this comparative study for helpful suggestions given during this investigation and the preparation of the manuscript.

Taxonomic studies of the *Penicillia* have necessarily been based for the most part on the imperfect stage. Few species complete their full life cycles in culture, and it will probably continue to be desirable as heretofore to prepare keys largely based upon the conidial apparatus so that those engaged in cultural and biochemical studies can recognize and identify the forms encountered. A few ascocarpic species have been described, but many of these

descriptions lack certain details which are essential to a better understanding of the group as a whole. Seventeen strains and twelve species of *Penicillium* and one species of *Byssochlamys* are included in this study.

During the early course of this study two lines of development seemed apparent. In one series of forms the perithecial wall was formed of interlacing hyphae which in some species were closely knit, but in others were loose or so scanty as to be hardly apparent. The ascocarp continued to expand, presumably by intercalary growth of the hyphae composing the wall so that the mature ascocarp might be many times the size it had attained when the first asci were formed. This series was further characterized by an end to end arrangement of the asci to form chains. The ascocarpic initials varied and the ascospores were marked in various fashions, although most were spiny.

The second series bore ascocarps which were sclerotium-like, the asci appearing in a cavity which formed at the center. The ascocarp in these forms reached a definitive size before the first asci appeared at its center and the thick-walled pseudoparenchymatous cells which made up the perithecial wall appeared to be incapable of further cell division. The asci in this series were borne on short stalks as side branches from the ascogenous hyphae. Occasional asci appeared end to end, but this was exceptional (**Dodge 1933**). In this series the ascocarp was initiated in a uniform manner at the crotch of a tree-like system of hyphae.

So sharply and completely were these two series differentiated that a generic separation was at first deemed advisable. However, the study of other species subsequently received from Dr. Thom indicates that the gap between the two can be bridged and has yielded further important information about this group of Ascomycetes. The information now at hand concerning the origin and development of the ascocarp in these forms gives a basis for a much better understanding of the genus *Penicillium*. It is still inadequate, however, for a revision of the genus or the creation of new generic names for the ascocarpic species. All species included in this study, even though they seem to follow divergent lines, are therefore referred to *Penicillium* unless they have been re-

ferred by their authors to some other genus. Likewise no revision of species is made, except that one form is described as new.

The salient features of the forms included in this study are shown in the diagram of text figure 16. It will be seen at once that most of the species under consideration fall into one of two groups, one group characterized by an ascocarp not at all sclerotized and enclosing asci borne in chains, the second by an ascocarp made up of pseudoparenchymatous tissue which, following the digestive activities of the ascogenous hyphae, subsequently contains stalked asci within a central cavity. In the first two species the structure which appears to function as an ascogonium is merely a slightly differentiated portion of the vegetative mycelium and is probably a structure simplified through reduction. In the next four species the ascogonium is highly differentiated. In the next six species a simple substitute type of ascogonium reappears but is borne upon a specialized system of branched hyphae, present also in the other species but in less well developed form.

The disposition of asci in chains would seem to be a character of considerable theoretical importance. Although it may have arisen as a reduced form from the more common type, it is strikingly different and a comparative examination of the asci alone of *Penicillium Wortmanni* and *P. Brefeldianum*, for example, would suggest that they belong not only in different species, but even in different families. On the basis of this and correlated differences the species first examined in this study were, as stated above, arranged in two distinct groups with nothing in common between them so far as the ascocarp was concerned. Later when *P. egyptiacum* was received and examined it was found that the nature of the ascocarp would place it in one group, while its asci resembled those of the other group. These twelve species can therefore be arranged, not in two unrelated groups, but along two divergent lines. *Byssochlamys fulva* does not seem to belong in either series.

However, the divergence that can occur within a genus is a problem requiring further study, and it can be solved only by a careful morphological study of the structures involved in reproduction. Until a large number of species have been thus studied it is unsafe to attempt revision of the genus *Penicillium*. This

study has tended to confirm Thom's group separation based on penicillial characters, but has shown some wide variations in reproductive structures within the biverticillate group. The final evaluation of these differences awaits examination of other forms. There is a greater range of variations among the Plectascales than within some other groups of Ascomycetes, and some of these differences appear to be important. For example, in *Thielavia terricola* there appears to be a crozier and nuclear fusion in the ascus, while in *T. sepedonium* neither is present (Emmons 1932). The loss of certain structures and the acquisition of new ones may occur with greater frequency than has been supposed.

Besides the variety of structures appearing within the genus certain variations are to be noted in some species. One of the most significant of these is the tendency toward loss of ascospore production. Within a few weeks after *Carpenteles asperum* had been received in this laboratory it had ceased to produce ascocarps in some subcultures. It was only from certain tubes, and from fertile sectors on cornmeal agar plate cultures that a fertile strain was recovered. *Penicillium vermiculatum* exhibited a similar tendency. In one of the two original strains fertility has in some measure been restored. The second now produces only conidia. Non-ascosporic strains have arisen repeatedly also in *P. Brefeldianum*. *P. egyptiacum* has also given a form predominantly conidial. These species are homothallic, as proved by many single spore isolations. Presumably, therefore, sterility is not caused by segregation of two complementary strains. This sheds some light on the problem of sterility (so far as ascospore production is concerned) in the many strictly conidial species of *Penicillium* in nature. Contrary to the data of Derx, the author has found not the slightest evidence of heterothallism in any of the species of *Penicillium* examined. *Penicillium avellaneum* is not included in this study because the available strain had become sterile. Probably, therefore, the great majority of species of *Penicillium* are non-ascosporic, not because they are haplonts, but because at the time some variation or mutation gave rise to the strain, or at a later time, there was a concomitant loss of fertility. Without attempting any explanation of the mechanism of this loss, we are yet justified in assuming that it occurs for such loss of fertility can

be observed in variants arising in the laboratory. It is reasonable to assume that the same forces operate in nature to produce strictly conidial strains of *Penicillium*.

The chains of asci merit some general description. Their arrangement in *Penicillium egyptiacum* seems to differ in no essential respect from that in the other forms, and since somewhat clearer preparations have been obtained of this species it will be used as the basis for a general description. Crushed aceto-carmine mounts have been the most useful method for study. In such mounts many of the groups of asci are either too compact and complex for an understanding of their relationships, or they are too much broken up. If properly made, however, aceto-carmine preparations show some short chains of asci so clearly that there can be no doubt of their arrangement. These chains consist usually of not more than 5 or 6 cells and of these, two or three will show ascospores in some stage of development (FIG. 9E).

The chains are spirally coiled, or at least curved, and in the younger cells the wall toward the center of the spiral is the more convex. In some cases the inner tip of the coil can not be clearly seen, in some cases it appears to be broken off, and in still others it may be slightly turned back to form a hook (FIG. 9D). In spite of its superficial resemblance to the organ which initiates ascus formation in many Ascomycetes, it is not a crozier, and careful search of the young asci has failed to reveal any vestige of such a structure. The asci are definitely end to end, and such an arrangement does not follow the intervention of a crozier of the conventional type. End to end arrangement might follow crozier formation if the ascus developed from the antepenultimate instead of the penultimate cell, but it does not seem necessary to postulate any such complicated sequence here. The ascus seems, rather, to develop directly from a cell of the ascogenous hyphae. It may be that some substitute for the crozier mechanism occurs earlier in the development. In the young ascogenous hyphae certain protuberances and hook-like structures bear a superficial resemblance to croziers, but it does not appear that they function in the manner of croziers.

The nuclear story for these *Penicillia* has not been elucidated, but all are homothallic. From 5 to 20 single spores, ascospores

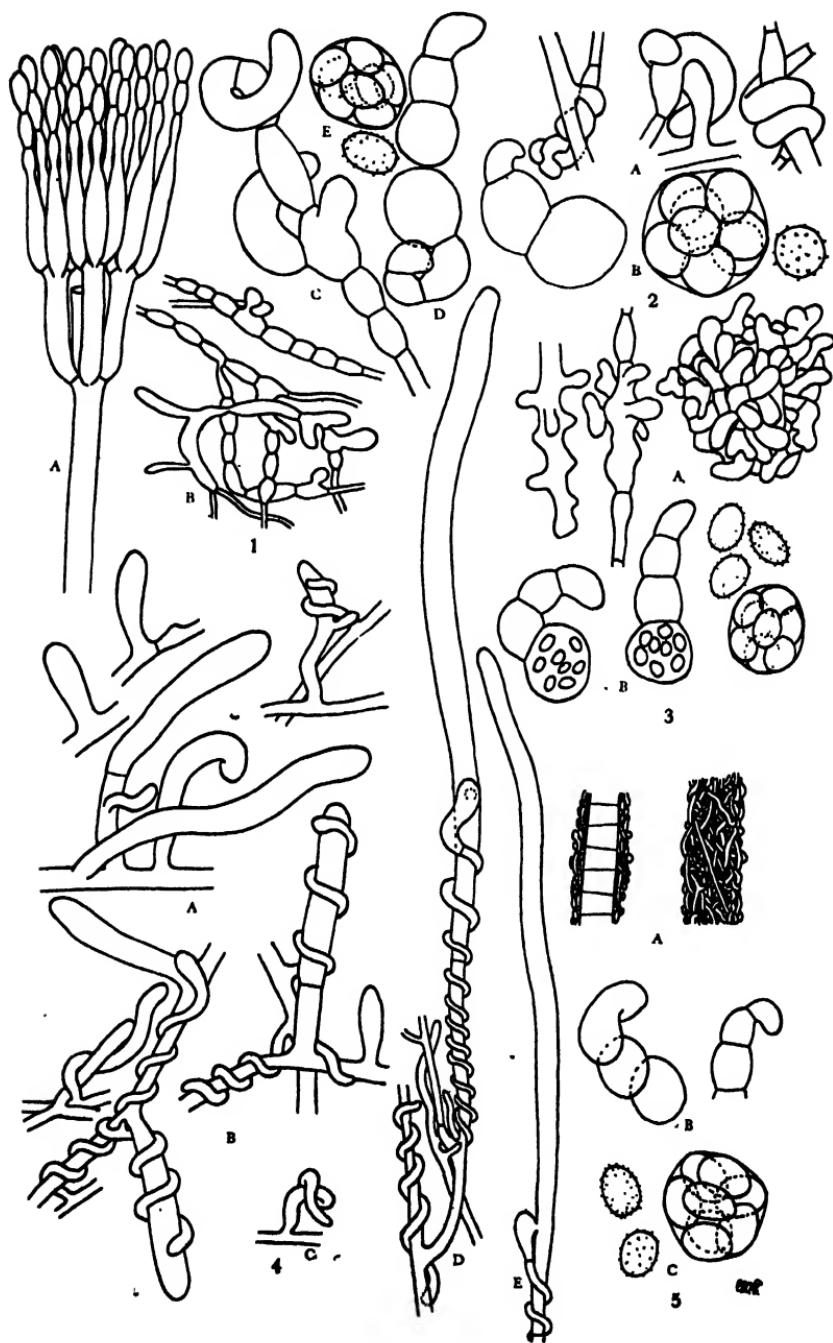
and conidia, have been isolated from each strain and in all species ascospores have been produced in such single spore cultures. As noted previously ascocarpic strains frequently lose the power to produce asci in culture.

Each of these 18 strains, with the exception of two, which are referred to a new species herein described, is typical of the species to which it is assigned, and seven are subcultures from the original strains described in papers cited. It will not be necessary, therefore, to give complete details of each one. It will be sufficient to consider each in turn and to point out some features, perhaps for the first time observed, which seem to be of phylogenetic significance.

*Penicillium Wortmanni* Klöcker 1903 (FIG. 1).

The ascocarp of *Penicillium Wortmanni* is very simple, like that of a *Gymnoascus*. There is no real perithecial wall. The ascogonium from which the ascocarp arises is also a simple structure, probably a reduced form. Its appearance is preceded by an increased vegetative development so that on cornmeal agar plates the ascogonia are to be found in hyphal tangles, which can be readily seen when one looks down upon the plate with the low power of the microscope. This hyphal development is particularly striking in forms in the second series considered here, and will be described in connection with those species.

Within these growths of aerial hyphae intercalary segments or side branches of the mycelium become swollen and cut up into short cells. In this condition they take up the stain more deeply (FIG. 1B, C). In most Ascomycetes, some change, conditioned by environment, age, and other factors, which we are accustomed to think of as maturity, is followed by the appearance of specialized structures, the ascogonia, and usually, antheridia. In *P. Wortmanni* a cell or slightly differentiated branch of the vegetative mycelium functions as an ascogonium, and no paired organs are to be found. This condition of maturity is not confined even to a single branch or to the progeny of its cells arising by cell division. It appears to spread to neighboring cells and to nearby hyphae. The growth of ascogenous hyphae is thereby initiated at many points within a region which in its later development assumes the character of a single ascocarp.

FIGS. 1-5. *Penicillium*.

The differentiated cells may be terminal or intercalary cells (FIG. 1B). Any given ascocarp may arise from one such plexus or from the fusion by centripetal growth of two or more such fertile regions. On cornmeal agar it is often possible to make out individual ascocarps with walls which are very loose in structure, but definitely specialized. On richer media the fertile regions are often quite extensive and limited only by the incrusted hyphae which surround them, without definite boundaries. The development of the perithecial wall is either very limited or lacking. Such a loose web of hyphae as is present is probably supplied by neighboring hyphae without any close connection with the ascogenous hyphae. The ascocarp is limited in its growth only by nutritional conditions. Paraffin sections as well as teased mounts show that ascii may develop while the fertile region is quite small, and that this fertile region or ascocarp increases in size by peripheral growth long after the first ascii appear.

The ascii are typically in chains (FIG. 1D). In exceptional cases ascii appear in this species and in others of this type, as side buds or terminal cells on a branch, but they are not ordinarily on stalks even in these cases. The terminal cell of a chain of young ascii at certain stages of development is often turned back upon itself (FIG. 1D). This suggests a crozier, although the exact relationship is very difficult to make out. This arrangement of ascii and the possibility of a crozier mechanism has been discussed above. The eight ascospores are spiny (FIG. 1E). This species was proved by the writer to be homothallic.

*Penicillium spiculisporum* Lehman 1920 (FIG. 3).

The strain of *Penicillium spiculisporum* coming from Porto Rico, upon which this study was based, has already been described (Kesten, et al. 1932). It seems to be typical of the species. The structure preceding the formation of the ascocarp is similar to that seen in *P. Wortmanni* (FIG. 3A), but is somewhat more specialized, and it arises in the midst of a branching hyphal system which is more highly developed in *P. egyptiacum* and will be described in connection with that species. Near the center of this loose vegetative hyphal system a side branch or an intercalary portion of a hypha swells, shows characteristic staining reactions, and branches profusely. These branches are short, gnarled, and form

a compact mass (FIG. 3A). From this hyphal knot develops an ascocarp varying greatly in size and possessing a well differentiated perithecial wall of interlacing hyphae of several cell layers in thickness. This definite perithecial wall does not, however, wholly restrict further growth of the ascocarp. The latter may increase in size during the formation of ascii. Presumably this growth is permitted by intercalary growth of the compact web of hyphae making up the wall. The eight-spored ascii in this form also are formed end to end. The ascospores are spinulose (FIG. 3B). The species is homothallic.

*Penicillium bacillosporum* Swift 1932 (FIG. 2).

The ascocarp of *P. bacillosporum* arises from a pair of short coiled hyphae (FIG. 2A). These are comparable to the primordia in *P. stipitatum* described below; but unlike that species the ascocarp develops directly around the primordium. The ascii, like others in this series, lie end to end to form short, curved or coiled chains. The eight spores are globose, and the walls are marked by comparatively coarse spines (FIG. 2B). The mature ascocarp of this species has been fully described by Swift (1932). The homothallic nature of this species was proved by the describer and was verified in the present study.

*Penicillium vermiculatum* Dangeard 1907 (FIG. 4 AND 5).

Two strains of this species have been studied. One was isolated from normal skin in a survey to determine the prevalence of yeast-like fungi in normal individuals (Benham and Hopkins, 1933). The second was received from Dr. Westerdijk under the label *Arachniotus ruber*. The rediscovery of the species at this time is of interest as confirmation of Dangeard's description, and its peculiar morphologic features make it a particularly interesting form in connection with the present study.

The ascogonium and antheridium of *P. vermiculatum* form a striking picture. Since Dangeard described it there have been few references to it. Derx (1925) identified a culture received from Thom with this species, but without giving any description of it. If examined at the time the ascogonia are forming it could not be mistaken for any of the other species included in this study. The ascogonium is a long clavate cell reaching an occasional length of  $250\mu$ . (FIG. 4A, B, D, E). The antheridium which Dangeard

calls the "trophogone" arises usually from a separate, more delicate hypha, and coils around the base of the ascogonium several times (FIG. 4B, D, E). An enlarged terminal cell is cut off and this comes into open communication with the ascogonium through a large pore (FIG. 4D, E). The various stages in this development and some of the less common branched forms which are found are shown in these figures.

Dangeard described and figured the early stages in ascocarp formation in this species in great detail. The primordia which he described are radically different from those described by Zukal, Brefeld and others who have studied *Penicillia* and from all other forms studied here. The very definite organ which he found fusing with the ascogonium was assigned to the rather indefinite function of serving as a "trophogone." While Dangeard admitted that this cell fusion occurred between the two organs he denied that there was any fertilization by a nuclear fusion. It is very gratifying to be able to confirm in many details Dangeard's report on the origin and development of the ascocarp in this species, although we are compelled to believe that there is an actual copulation, leading to sexual reproduction; or, in other words, Dangeard's "trophogone" is, in fact, a true antheridium.

The ascogonium becomes septate and enveloped in closely wound delicate hyphae (FIG. 5A). According to Dangeard's report most of the isodiametric cells in the upper three-fourths of the ascogonium give rise to ascogenous hyphae. The young ascocarp is consequently at first club-shaped and then elliptical. Dangeard states that mature ascocarps are elliptical. In our cultures on cornmeal agar plates the ascocarps finally become spherical and reach a diameter of 1 mm. The ascocarp has a definite wall several cell layers in thickness. The asci are borne in chains and are eight-spored (FIG. 5B, C). Dangeard figures asci with not more than six spores, but states in the text that the asci are eight-spored. After being in culture for several months the first of these strains became less fertile and produced smaller ascocarps. In cultures from single ascospores the fertility has been in some measure restored. This species as well as the others studied, is homothallic; single ascospores readily produce ascospores again. The ascospores are spiny (FIG. 5C).

**Penicillium stipitatum** Thom sp. nov. (FIG. 6 AND 7).

Mycelio homothallico; perithecio globoso, 130–400  $\mu$  diametro; ascis ovoideis, 5.5–6.5  $\times$  7–8  $\mu$ , 8-sporis; ascosporis ellipticis, 2–2.2  $\times$  3–3.6  $\mu$ , marginato.

Colonies on Czapek's solution agar floccose tufted in yellow (luteus) shades passing over to orange or even red orange shades in age; reverse yellow to red orange; aerial hyphae studded with granules yellow in the young and growing period becoming reddish in age; conidial apparatus irregularly biverticillate with sterigmata up to 10 by 2  $\mu$  taper pointed and closely packed in the verticil; conidia about 3.5  $\mu$  in long axis, rather thick walled fusiform smooth; ascogenous masses enveloped by tufts and masses of yellow hyphae, within which hyphae are arranged into a fairly definite wall forming a brittle and easily crushed peritheciun; asci 8-spored, ripening quickly, 5.5–6.5  $\times$  7–8  $\mu$ .

Ascospores 3–3.6  $\mu$  in long axis by about 2  $\mu$ , lens-shaped consisting of a two valved body with an equatorial band or frill about 0.5  $\mu$  in width, usually appearing single but occasionally apparently double with a groove partially evident between them.

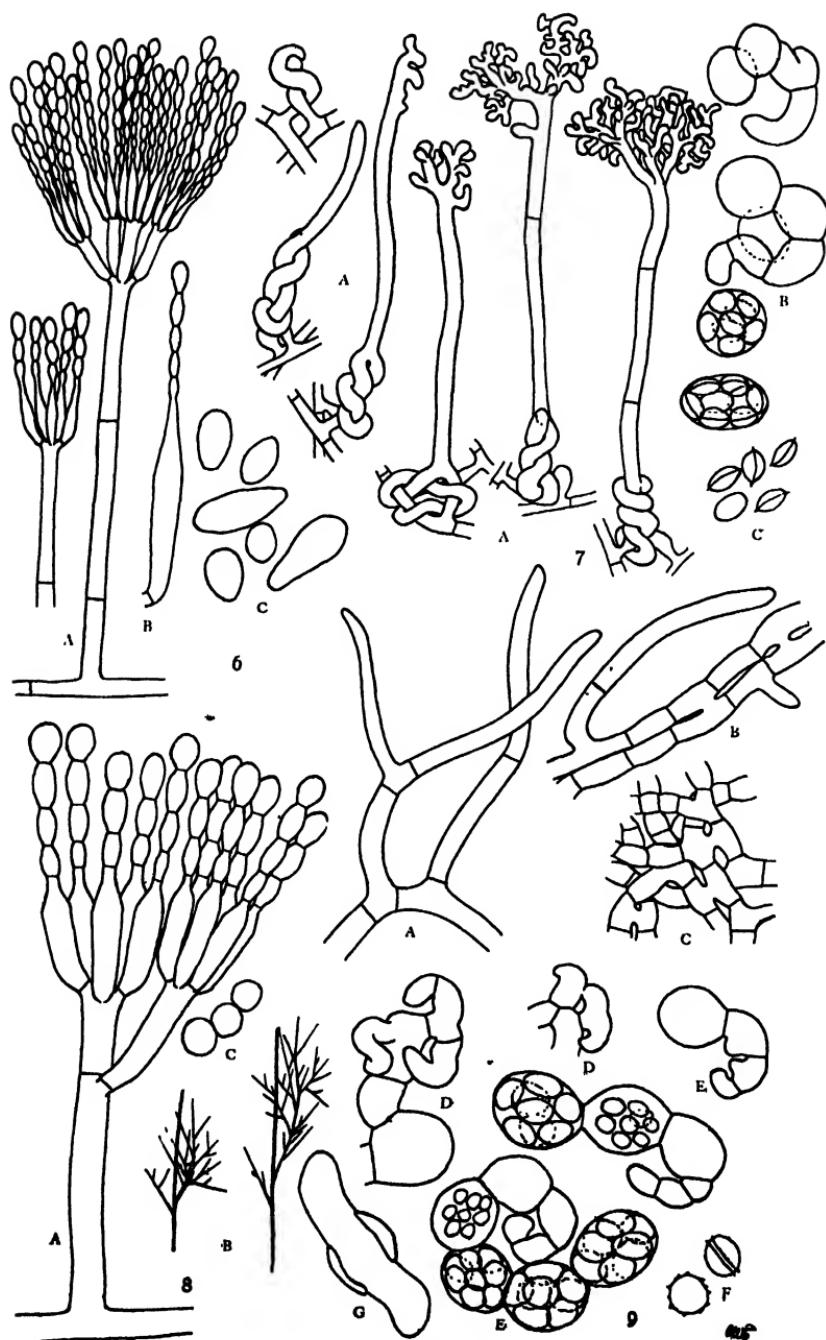
Cultures have been deposited with the American Type Culture Collection and with the Centraalbureau voor Schimmelcultures, Baarn.

Two strains of this fungus have been studied. One was isolated from rotting wood in Louisiana and was sent to Dr. Thom's laboratory by T. C. Sheffer. The second was sent to him by Dr. Ma from Peiping, China. Because of distinctive features which could not be identified with any other *Penicillium* this form is described as a new species.

The two strains of this fungus which have been studied vary slightly in growth habit and in some measurements. They are too nearly alike, however, for specific differentiation. The second produces larger ascocarps and more conidia. Conidia in both strains are formed sparingly. The elliptical conidia are borne on biverticillate penicilli (FIG. 6A). The sterigmata are long and tapering, as in others of this group (FIG. 6B). The ascocarpic initial consists of a pair of short hyphae which complete one or two coils about each other and fuse by a large permanent pore. The resulting hypha elongates to about 100  $\mu$  and the ascocarp

forms at its tip (FIG. 7A). The perithecial wall is composed of interlacing hyphae which do not become pseudoparenchymatous. The 8-spored asci are borne in chains (FIG. 7B). Each ascocarp is encircled longitudinally by a flange (FIG. 7C).

The ascocarpic primordium of *Penicillium stipitatum* is a most remarkable structure, and, so far as known, is without parallel in other forms. In *Aspergillus herbariorum*, according to Kny and DeBary, the mature ascocarp is raised upon a single hyphal thread support, but here the ascocarp originates from primordia developed at the top of this hyphal stipe instead of at the base as in *Penicillium stipitatum*. Furthermore, in *Aspergillus* the stalk supports the mature ascocarp, while in *Penicillium stipitatum* the cells of the stipe become vacuolate, and the ascocarp is supported, rather, by surrounding vegetative hyphae. In such forms as *Sclerotinia* where the ascogonial coil is developed in the sclerotium, the ascocarp is supported on a stipe, which is, however, composed of many hyphae, some of which, at least, are gametophytic. In *Penicillium stipitatum* two similar or barely differentiated hyphae arise as side branches, usually from different hyphae, and coil around each other (FIG. 7A). After about two turns they fuse by a large pore so that the opening is of a diameter equal to that of the inside of the hypha. This opening is permanent. The two branches that initiate this development are without doubt the ascogonium and the antheridium. From our knowledge of what takes place in other fungi we may assume that fertilization takes place at this point. Cytological proof in this case has not yet been obtained. We would now expect the development of an ascocarp around this structure as in other *Penicillia*. The actual development is quite different. One of the branches, or the hypha arising from the union of the copulating branches, elongates until it reaches a length of 100–150  $\mu$  (FIG. 7A). It becomes once or twice septate, and at its tip begins to put out branches. These gnarled branches are formed in profusion, become septate, and by their further branching and intertwining, form a more or less compact mass not unlike the ascocarpic initial of *P. spiculisporum*. Within this ascocarp ascogenous hyphae appear and give rise to asci arranged end to end in chains (FIG. 7B).

FIGS. 6-9. *Penicillium*.

The ascospore of *Penicillium stipitatum* is also remarkable. It is surrounded by a flange which is very delicate and extends some distance beyond the spore wall (FIG. 7C). In a few exceptional cases two parallel rings appear to be present. The spores lie in the ascus in such a manner that the flange is parallel to the ascus wall. Cytological preparations, while not entirely satisfactory, do show, at an earlier stage, the eight nuclei of the ascus distributed through the cell and arranged so that their beaks radiate toward the ascus wall. The flange may be an extension of the kinoplasm, and at one stage possibly forms a continuous spherical membrane inside of and parallel to the ascus wall. The flange is not a surface configuration which arises after the spore is otherwise fully matured, but in stained sections shows early in spore development.

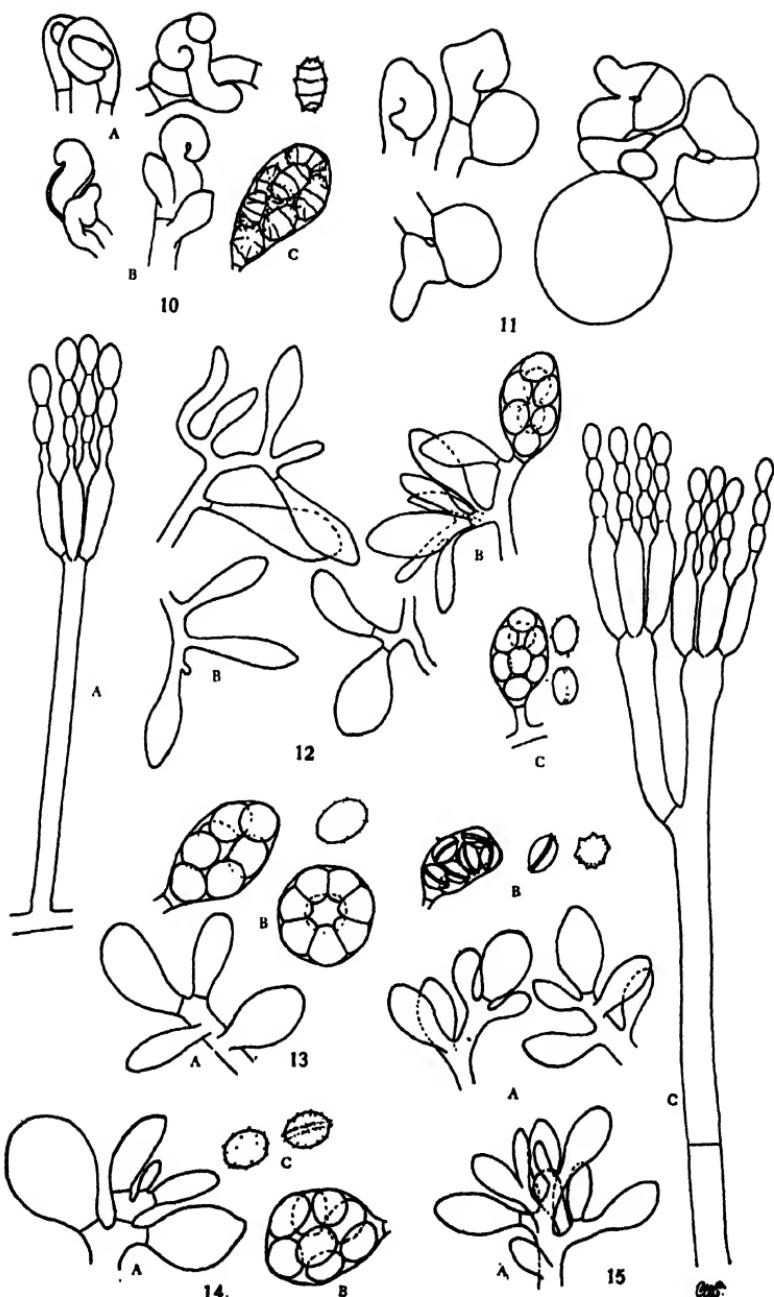
*Penicillium stipitatum* is homothallic as proved by numerous single spore isolations.

*Penicillium luteum* Zukal 1889 (FIG. 10).

This strain was sent from Manitoba by Dr. Bishy to Dr. Thom. It is apparently the species described by Zukal since it conforms to the description he gave.

The ascocarpic initial is composed of a pair of short coiled hyphae (FIG. 10A). These complete one or two turns and the ascocarp develops directly around them. More than one pair of initials may become involved in the formation of one ascocarp. The perithecial wall is composed of a very loose weft of hyphae. The ascocarp continues to increase by peripheral growth and by the accretion of adjacent young ascocarps after the first asci appear. The fertile regions of a colony, which are bright yellow, as in *P. Wortmanni* are covered by specialized encrusted hyphae. In *P. luteum* these hyphae are wavy and sometimes spirally coiled.

In spite of its superficial resemblance to *P. Wortmanni* an examination of the young asci of *P. luteum* reveals a very different organization. While the asci of the former species are borne end to end, those of the latter are borne as sessile side buds from an ascogenous hypha. Their appearance is therefore very different also from that in the *P. Brefeldianum* series since the conspicuous stalks are absent. The arrangement of the ascogenous hyphae and the young asci is to some extent obscured by a brown pig-

FIGS. 10-15. *Penicillium*.

mentation in these structures and by a layer of gelatinous material associated with the walls. It appears, however, that croziers are present (FIG. 10B) and that ascii arise through them.

The development and nature of the ascus suggests that *P. luteum* is not closely related to the other forms studied here. The extreme reduction in its conidial apparatus also suggests this view. Many of the fractional penicilli are reduced to single cells bearing a chain of spores. It may be, however, that when other forms are found and studied some species will be found to bridge the gap here as was the case in the two other types of ascus arrangement which seemed at first fundamental. Derx (1925) states that this species is heterothallic. Our single spore cultures have produced abundant ascospores. This evidence, and the fact that several other homothallic species of *Penicillium* may become non-ascosporic in culture, indicate that Derx probably encountered, not heterothallism, but some type of variation.

*Penicillium egyptiacum* van Beyma 1933 (FIG. 8 AND 9).

Two strains of this species have been available, presumably from the same original source. One was sent to Dr. Thom from Dr. Westerdijk; the second was transmitted to us by Dr. Thom as van Beyma's type. Because it combines some of the characters of the two types of *Penicillium* outlined above, and because it corresponds in so many respects with the fungus described by Brefeld as *P. glaucum* this is probably the most important species included in this study.

The penicillus of *Penicillium egyptiacum* is monoverticillate or sometimes asymmetrically biverticillate, and it bears the short, abruptly tapering sterigmata which characterize this series of forms (FIG. 8A).

The ascocarpic initial of *Penicillium egyptiacum* is like that described by Dodge for *P. Brefeldianum*. The details of its structure can be made out more easily here than in some other species. When this fungus is grown on cornmeal agar plates at 30° very few conidia are produced, and there is a good production of ascocarps. When an appropriate zone near the edge of a colony grown under such conditions is examined under the low power of the microscope the only aerial hyphae to be seen may be these ascocarpic initials. They appear as upright branching

systems arising from large hyphae in the substrate (FIG. 8B). If some of this material is mounted in aceto-carmine the branches making up this system take up the stain more deeply than the other hyphae. They are long, slender and somewhat antler-like (FIG. 9A). Transverse septa are at first far apart. At a later stage these branches, and particularly the inner ones, stain more deeply with aceto-carmine, numerous cross septa appear, many branches develop, and hyphal anastomoses are frequent (FIG. 9B, C). The hyphal anastomoses may be found in other parts of the mycelium, but they are more numerous in this region and it seems probable that they are of some significance in ascus production. The ascocarp now rises directly from the center of this hyphal plexus. There are no paired organs, but the repeated branching and intertwining of these hyphae, now cut up into short cells, form a compact mass of pseudoparenchymatous cells. Young ascogenous hyphae resembling those figured by Brefeld, appear in a few days at the center of the ascocarp (FIG. 9D). The peculiar type of branching these assume is distinctive. Cell fusions can sometimes be found, but these are not constant features. Despite the crooked contorted nature of these hyphae and the chain of asci into which the cells develop, the sequence can sometimes be made out (FIG. 9E). The asci are certainly arranged end to end, although they are often somewhat coiled. The ascospores, eight to an ascus (contrary to van Beyma's account), are surrounded by a longitudinal furrow bordered by flanges (FIG. 9F). Upon germination they swell enormously and split along the furrow (FIG. 9G).

In its type of ascocarp, in the character of its ascogenous hyphae, in the arrangement of asci in chains, and in type of ascospore *Penicillium egyptiacum* is like *P. glaucum* of Brefeld. Our strains, however, do not have the type of ascogonium Brefeld figured, they produce asci promptly, and they do not produce typical apple rot. The ascospore markings are perhaps less conspicuous. *P. egyptiacum* is more nearly like Brefeld's fungus than is *Carpenteles asperum* which has recently been put forward as that species. In any case we now know that Brefeld must have been correct in describing a *Penicillium* with a sclerotium-like ascocarp enclosing asci disposed in chains, and ascospores with

longitudinal furrows splitting on germination like those of *Aspergillus*.

Single spore isolations have proved that this species is homothallic.

*Penicillium Ehrlichii* Klebahn 1930 (FIG. 14).

This strain was subcultured from Klebahn's type. Its ascogonium and ascocarp are of the same type as those of *P. egyptiacum*, but its asci are borne as side branches from the ascogenous hyphae (FIG. 14A). It is, then, a representative form in the second series of ascus-bearing *Penicillia* except that the ascocarp in *P. Brefeldianum* and *Carpenteles asperum* become much harder. The ascocarpic initial of *Penicillium Ehrlichii* is like that of *P. egyptiacum* but the hyphal branches are somewhat longer and more delicate. The differentiation of the hyphae at the center of this hyphal system appears to follow the same order in the two species. The ascospore of *P. Ehrlichii* shows a longitudinal furrow bordered by prominences and the entire surface is marked by spines (FIG. 14C).

The homothallic nature of *Penicillium Ehrlichii* was proved by numerous single spore isolations.

*Penicillium Brefeldianum* Dodge 1933 (FIG. 13).

The ascogonium of *Penicillium Brefeldianum*, as Dodge pointed out, arises in the crotch of a tree-like growth of hyphae. The exact morphological structures at this point are very difficult to make out. The innermost branches of this system seem to swell and to take on characteristic staining properties as in the less differentiated hyphae of *P. Wortmanni*. In some, and perhaps all, of the forms of this type there are hyphal anastomoses in this pleux (see *P. egyptiacum*). From some of these structures, which may function as primary ascogenous hyphae so well described by Brefeld, the ascogenous hyphae arise; from others the sterile tissue which surrounds the ascogenous hyphae. The eight-spored asci are borne on stalks (FIG. 13A, B). The species is homothallic. The further development of this form has been fully described by Dodge (1933).

*Penicillium javanicum* van Beyma 1929 (FIG. 12).

The development of two strains studied is as given by van Beyma (1929) and Dodge (1933). One of these is van Beyma's

original strain and the other was sent to Dr. Thom by Dr. Ma from Peiping, China. Both are 8-spored but correspond otherwise with van Beyma's description. In all respects save measurements, color, and degree of sclerotization this species is much like *P. Brefeldianum*. The ascospores form on stalks (FIG. 12B). Under the proper conditions of lighting and magnification a thin region can be made out extending around many of the spores in a manner to suggest a valve (FIG. 12C). This band is not, however, bordered by wings. Both strains are homothallic.

*Carpenteles asperum* Shear 1934 (FIG. 15).

This strain has been recently described by Shear (1934). He considered this to be the form Brefeld studied (1874) and the title of his article reads, "*Penicillium glaucum* of Brefeld (*Carpenteles* of Langeron) refound." However, because of uncertainties regarding Link's type he proposed a new name, *Carpenteles asperum* adopting the generic name proposed by Langeron (1922). Shear reconciles the incomplete agreement between this form and that described by Brefeld by the statement, commonly accepted, that Brefeld was working with impure cultures. Whether Brefeld's cultures were pure or impure may never be known, but there is now good evidence at hand for believing that his story of the development of "*Penicillium glaucum*" is correct, save perhaps in the matter of the primordium and conidia. This evidence is supplied by an examination of *P. egyptiacum*. While *P. egyptiacum* is not actually the species described by Brefeld, it at least coincides so closely with it that the facts reported here amount to a virtual verification of Brefeld's data.

The ascogonium of *Carpenteles asperum* arises as in the preceding forms in the crotch of a system of hyphal branches, and the early development is similar to that of *P. Brefeldianum*. The sclerotium-like ascocarp becomes so hard, however, at certain stages, that it is very difficult to crush it under a cover slip, and good paraffin sections are almost impossible to secure. Ascospores do not usually appear for several weeks. The ascospores present the double wing described by Brefeld (FIG. 15B). The ascospores are however, formed on stalks (FIG. 15A) rather than in chains. Single spore isolations prove the species to be homothallic.

*Penicillium Gladioli* Machacek 1927.

species	ascogonium	young ascocarp	asci	ascospore
<i>Penicillium Wortmanni</i>				
<i>P. spiculisorum</i>				
<i>P. bacillosporum</i>				
<i>P. vermiculatum</i>				
<i>P. stipitatum</i>				
<i>P. luteum</i>				
<i>P. egyptiacum</i>				
<i>P. Ehrlichii</i>				
<i>P. javanicum</i>				
<i>P. Brefeldianum</i>				
<i>Carpenteles asperum</i>				
<i>Penicillium Gladioli</i>				(The sterile sclerotium of <i>P. Gladioli</i> in its early stages resembles the ascocarp in this series of forms).
<i>Byssochlamys fulva</i>				

FIG. 16. Diagrammatic representation of the ascocarpic structures in *Penicillium*.

A strain of *Penicillium Gladioli* is included for comparison although no ascii have been found in our cultures. The abundant sclerotia remain sterile. This strain was isolated by the authors from a *Gladiolus* corm. The primordium of the sclerotium arises in the crotch of a tree-like system such as Dodge described for *P. Brefeldianum*. The subsequent development also is like that species except that ascii do not appear at the center of the sclerotium. The sclerotia of *P. Thomii* and similar forms have not been studied.

*Byssochlamys fulva* Olliver and Smith 1933 (FIG. 11).

*Byssochlamys fulva* differs widely from other forms studied here in conidial as well as ascocarpic structures. The conidiophore is that of the *Paecilomyces varioti* series. The ascocarp lacks a perithecial wall and the ascii are borne in clusters, forming tiny white flecks, easily seen among the brown conidia. These ascii are preceded by the formation of a conspicuous crozier (FIG. 11) so that the young ascogenous hyphae of this species are very different in appearance from those of the other forms studied here. *Byssochlamys fulva* does not fit into either of the series outlined here.

The fungus is homothallic but there is a tendency toward sterility in strains propagated by either mass or single spore transfers.

#### SUMMARY

A morphologic study of the reproductive structures involved in the ascomycetous phase of 12 species (17 strains) of *Penicillium* and of one related species has revealed a surprising diversity in ascogonia and antheridia, in types of ascocarps, in the disposition of the ascii, and in ascospore markings. Two divergent lines appear, but there are species having some of the characters of each line. The ascocarpic initials of *Penicillium Wortmanni* and *P. spiculisporum* are barely differentiated segments of the vegetative hyphae. Those of *P. bacillosporum* and *P. luteum* are paired coiled organs. In *P. stipitatum*, described as a new species, the fusion of the ascogonium and antheridium results in the formation of a hypha 100–150  $\mu$  long at the tip of which the ascocarp forms. The ascogonium of *P. vermiculatum* is a clavate cell, reaching a length of 250  $\mu$ , around the base of which the antheridium coils. In *P. egyptiacum*, *P. Ehrlichii*, *P. javanicum*, *P. Brefeldianum*,

and *Carpenteles asperum*, the young ascocarp arises from the innermost branches of a tree-like system of branches. *Penicillium Gladioli* represents the sterile sclerotium forming species. *Byssochlamys fulva* has a short hyphal coil from which the ascogenous hyphae arise, and through the intervention of croziers naked ascii develop. In the first seven species the ascii are in chains and in the next four they are on stalks. All species studied are homothallic.

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#### EXPLANATION OF FIGURES

Fig. 1, *Penicillium Wortmanni*: A, Conidiophore.  $\times 1300$ ; B, Ascogonia.  $\times 500$ ; C, Ascogonium.  $\times 1300$ ; D, Chains of young ascii.  $\times 1300$ .

Fig. 2, *Penicillium bacillosporum*: A, Ascogonia and antheridia; B, Ascii in chains, a mature ascus, and ascospores.  $\times 1300$ .

Fig. 3, *Penicillium spiculisorporum*: A, Ascogonia and young ascocarps; B, Ascii in chains, a mature ascus, and ascospores.  $\times 1300$ .

Fig. 4, *Penicillium vermiculatum*: A, Young ascogonia.  $\times 1300$ ; B, Young ascogonia and antheridia.  $\times 1300$ ; C, Young antheridium.  $\times 1300$ ; D, Mature ascogonium and antheridium.  $\times 500$ ; E, Mature ascogonium and antheridium in profile.  $\times 500$ .

Fig. 5, *Penicillium vermiculatum*: A, Portion of a very young ascocarp in optical section and surface view; B, Chains of young ascii; C, Mature ascus and ascospores.  $\times 1300$ .

Fig. 6, *Penicillium stipitatum*: A, A monoverticillate and a biverticillate conidiophore.  $\times 500$ ; B, A sterigmata.  $\times 1300$ ; C, Conidia.  $\times 1300$ .

Fig. 7, *Penicillium stipitatum*: A, Stages in the development of the ascogonia.  $\times 500$ ; B, Chains of young ascii.  $\times 1300$ ; C, Mature ascii and ascospores, the spores shown in both views.  $\times 1300$ .

Fig. 8, *Penicillium egyptiacum*: A, Conidiophore.  $\times 1300$ ; B, Habit sketch of the vegetative development preceding appearance of the ascocarp.  $\times 100$ ; C, Mature conidia.

Fig. 9, *Penicillium egyptiacum*: A, Hyphae in the primordium of the ascocarp; B, Hyphal fusions in the ascocarp primordium; C, A later stage in the development of the primordium; D, Ascogenous hyphae; E, Chains of ascii; F, Ascospores, face and side views; G, Germinated ascospore.  $\times 1300$ .

Fig. 10, *Penicillium luteum*: A, Ascogonia and antheridia; B, Ascogenous hyphae; C, Mature ascus and ascospore.  $\times 1300$ .

Fig. 11, *Byssochlamys fulva*, ascogenous hyphae and young ascii.  $\times 1500$ .

Fig. 12, *Penicillium javanicum*: A, Conidiophore; B, Young stipitate ascii; C, Mature ascus and ascospore.  $\times 1300$ .

Fig. 13, *Penicillium Brefeldianum*: A, Young ascii; B, Mature ascii and ascospore.  $\times 1300$ .

Fig. 14, *Penicillium Ehrlichii*: A, Young ascii; B, Mature ascus; C, Ascospores.  $\times 1300$ .

Fig. 15, *Carpenteles asperum*: A, Young ascii; B, Mature ascus and ascospore; C, Conidiophore.  $\times 1300$ .

Fig. 16, Diagrammatic representation of the ascocarpic structures in *Penicillium*.

# THE GENUS DICHEIRINIA<sup>1</sup>

GEORGE B. CUMMINS

(WITH PLATE 16 AND 1 TEXT FIGURE)

The genus *Dicheirinia* was established in 1907 by Arthur (1) with *Triphragmium binatum* Berk. & Curt. as the type and only species known. Both uredia and telia were known at that time but the available material was too scanty to allow a detailed study. In 1925, when part 10 of volume 7 of the North American Flora was published, Arthur reported numerous other collections but considered to bear only uredia and telia.

Jackson (5) in 1931 added a second species, *D. superba* Jacks. & Holw., and made a substantial contribution to the knowledge of the genus by showing that subcuticular pycnia consistently occurred with the microtelia. The present study adds two species by transfer from other genera, confirms Jackson's discovery of pycnia, shows *D. binata* to be macrocyclic with uredinoid aecia and offers an emended description of the genus to accord with these facts.

## PYCNIA AND AECIA

The pycnia are subcuticular, as pointed out by Jackson (5), and occur rather abundantly among the aecia in *D. binata* and among the microtelia in *D. superba* and *D. manaosensis*. Development of the following spore form often obliterates all trace of the preceding pycnia and doubtless accounts for the tardy recognition of the kind of life cycle.

Uredinoid aecia are known only in *D. binata* but are of common occurrence. In the Arthur Herbarium the finest development of aecia is shown on *Erythrina Crista-galli* L., collected July 7, 1924 at the Agricultural Experiment Station, Mayaguez, Porto Rico by Whetzel, Kern and Toro as no. 2415.

Discal paraphyses like those in the uredia are present in the aecia but are less abundant and may be scarce. The aecia are

<sup>1</sup> Contribution from the Botany Department, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

borne on distorted and hypertrophied veins and petioles or less commonly on the leaf blade. There being no peridium growth is rather indeterminate and may be locally systemic.

#### UREDIA AND TELIA

Subepidermal uredia are present in *D. binata* and *D. Ormosiae* and offer peculiarities in structure of paraphyses worthy of note. Those of *D. binata* are discal while in *D. Ormosiae* they are peripheral. The paraphyses are unevenly knob- or clove-like in *D. binata* (PLATE 16, FIG. 3) and while specifically distinct easily recall the paraphyses described in other genera. Those of *D. Ormosiae*, however, are unique in so far as the writer is aware. The terminal portion, corresponding to the head in most paraphyses, is profusely branched and dendritic with the ultimate branches tubercle- or finger-like (PLATE 16, FIG. 4). The degree of subdivision varies but may be great. A sorus encircled by these tangled structures appears to be possessed of a peridium (TEXT FIG. 1), and is as sharply limited in extent as though a peridium were present. In young sori the spores are completely covered by the mass of paraphyses but with maturity are liberated through a pore-like opening formed by the upward and outward development of the paraphyses.

Telia are subepidermal with paraphyses as in the uredia. In the macrocyclic species the sori are small and scattered but in the microcyclic species simulate the habit of aecia, occurring on hypertrophied areas among the pycnia and, in *D. superba*, covering extensive areas and apparently arising from a locally systemic mycelium as indicated by Jackson (5). While the material is too scanty to allow a definite statement the same development seems likely in *D. manaoensis*.

Sculpturing on the teliospores, while differing in the various species, shows a marked similarity in all (PLATE 16, FIG. 1, 5, 7, 9) being coarsely cubical or digitate and more numerous above. The structure of the pedicel provides the character most distinctive of the genus. The basal stalk is relatively long and fragile, early collapsing to liberate the spores. Shortly below the junction of the pedicel and the spores a horizontal septum is formed dividing the pedicel into a lower and an upper portion. The upper portion

may consist of a single cell (*D. Ormosiae*, PLATE 16, FIG. 5, 6), of two cells (*D. binata*, PLATE 16, FIG. 1, 2; *D. superba*, PLATE 16, FIG. 9, 10) or of three cells (*D. manaosensis*, PLATE 16, FIG. 7, 8) to which the teliospores are directly attached. The pedicel breaks at or below the horizontal septum leaving the teliospores, when mature, carrying only the apical cells of the pedicel. These may collapse to such an extent that no indication of a pedicel remains on mature spores.

Each teliospore is provided with one apical germ pore placed next the vertical walls separating the spores. The sculpturing of the spore wall often makes observation of the pore difficult and in spite of careful study its location in *D. Ormosiae* remains doubtful. This uncertainty offers the only substantial obstacle to the ready acceptance of this species as a member of the genus.

#### RELATIONSHIPS OF THE GENUS

In naming the genus Arthur (1) placed it in the tribe Ravenelliae of the Aecidiaceae and Dietel (4) treated it in the same manner, assigning it to the tribe Raveneliae of the family Pucciniaceae. According to Dietel's scheme the genus merits a position between *Diabole* and *Uromyctadium* and near *Sphenospora* and *Diorchidium*.

In a study of the genera probably most closely related to *Dicheirinia* one is impressed by the similarity of the teliospores of species of *Diorchidium* and of *Hapalophragmum*. *Diorchidium* differs in having no apical cells on the pedicel and in most species by a lateral rather than an apical placement of the germ pores. *Diorchidium Piptadeniae* has the pores apical and except in possessing a continuous pedicel is similar to *Dicheirinia superba*. *Dicheirinia manaosensis* appears much like *Hapalophragmum setulosum* and *H. ponderosum* in that three cells are borne together. In *Hapalophragmum*, however, the pedicel is continuous and bears a 3-celled teliospore with two cells joined to the pedicel and the third superimposed upon the basal pair. The similarity between *Dicheirinia* and *Hapalophragmum* is perhaps more apparent than real.

In the possession of an apical cell in the teliospore pedicel *Dicheirinia* closely simulates *Diabole*, but differs in other charac-

ters. The latter genus has a pedicel usually bearing several pairs of teliospores, each pair attached to a single apical cell of the pedicel but the spores are more definitely independent of each other and have pores probably lateral. Despite careful study the location of the pores could not be definitely established. It seems probable that there are two pores in each cell, somewhat below the equator, one at the inner angle, the other opposite in the outer wall. If this assumption proves correct the differences between *Dicheirinia* and *Diabole* are considerable. The sori are subepidermal in *Dicheirinia* and subcuticular in *Diabole*. The writer believes the relationship to be more remote than the discussion by Dietel (3) would indicate.

The frequent occurrence of a 2-celled condition in the teliospore-heads of *Ravenelia simplex* Diet. offers an opportunity for comparison. The association of the two cells, the distribution of sculpturing and especially the structure of the pedicel, as given by Dietel (2, PLATE 6, FIG. 20C), parallel the same characters in *Dicheirinia*. As Dietel (2) has pointed out the presence of the several-celled heads gives the only indication of its generic position. The 2-celled condition in *R. simplex* is typical of that found in *Dicheirinia superba*.

While Dietel (4) is justified in indicating a close relationship to *Diabole* and *Uromycladium* the writer believes that the situation would be more correctly depicted by considering that *Dicheirinia* in its simpler condition (*D. Ormosiae*) closely approaches such a species as *Diorchidium Piptadeniae*. The insertion of a single apical cell in the teliospore pedicel of *D. Ormosiae* offers the only obstacle to its inclusion in *Diorchidium*. In its more complex species *Dicheirinia* seems definitely related to the genus *Ravenelia* through such a simplified condition as that mentioned above for *R. simplex*. Dietel (3) has previously pointed out the correspondence between the apical cells of the pedicel in *Dicheirinia binata* and the cysts in *Ravenelia*.

#### TAXONOMIC TREATMENT OF THE SPECIES OF DICHEIRINIA

##### DICHEIRINIA Arthur N. Am. Flora 7: 147. 1907.

*Pycnia* subcuticular, conic, hemisphaeric or crust-like, without paraphyses. *Aecia* when present subepidermal, uredinoid, without

peridium but with few to many paraphyses; aeciospores borne singly on pedicels, the walls colored and echinulate, pores distinct. Uredia when present subepidermal, with paraphyses; urediospores borne singly on pedicels, the walls colored and echinulate, the pores one or more, basal or equatorial, distinct. Telia subepidermal, with few or many paraphyses; teliospores two or three, united laterally on a common pedicel, the walls colored and coarsely tuberculate or digitate, with one apical pore, the pedicels having 1–3 apical cells.

Type species, *Triphragmium binatum* Berk. & Curt., on an undetermined plant (*Erythrina?*).

Arthur's definition of the genus is no longer adequate to care for the species which are here included. The discovery of pycnia and aecia necessitates an expansion of the generic characters. Microcyclic and macrocyclic species must both be considered. The presence of three teliospores in *D. manaosensis*, the consequent three apical cells in the pedicel and the fact that *D. Ormosiae* has only one apical pedicellate cell have all to be provided for. The above emended generic description is presented in light of present knowledge.

The genus is limited to the tropical regions of North and South America.

#### KEY TO THE SPECIES OF DICHEIRINIA

Teliospores borne two on a pedicel.

Teliospore-pedicel with one apical cell; macrocyclic.

Teliospore-pedicel with two apical cells.

1. *D. Ormosiae*.

Teliospore-sculpture digitate; macrocyclic. 2. *D. binata*.

Teliospore-sculpture cubical; microcyclic. 3. *D. superba*.

Teliospores borne three on a pedicel; microcyclic. 4. *D. manaosensis*.

#### 1. *Dicheirinia Ormosiae* (Arth.) comb. nov.

*Puccinia Ormosiae* Arth. Mycologia 9: 78. 1917.

*Dicaeoma Ormosiae* Arth. N. Am. Flora 7: 391. 1920.

Pycnia and aecia unknown. Uredia hypophylloous, scattered or more or less grouped, at first whitish from the mass of paraphyses, later chestnut-brown, surrounded by a dense peripheral rim of whitish paraphyses, appearing peridium-like (TEXT FIG. 1), paraphyses profusely branched and expanding into a dendritic or botryoid head (PLATE 16, FIG. 4), large, colorless but refractive to light; urediospores irregular, obovoid with the pore in face

view, triangular with pore in lateral view, 20–26 by 24–32  $\mu$ ; wall dark cinnamon-brown, 1.5  $\mu$  thick, somewhat thicker at hilem, sharply and sparsely echinulate, pore one, near the hilem, distinct. Telia hypophylloous, grouped like the uredia but chocolate-brown, paraphyses as in the uredia; teliospores (PLATE 16, FIG. 5, 6) one-celled, borne in closely united pairs (rarely 3) on a common pedicel, oblong or ellipsoid, flattened on the inner side, 15–20  $\mu$  wide by 25–32  $\mu$  high; wall chocolate-brown and nearly opaque, 3–4  $\mu$  thick including the tubercles, coarsely tuberculate with ir-

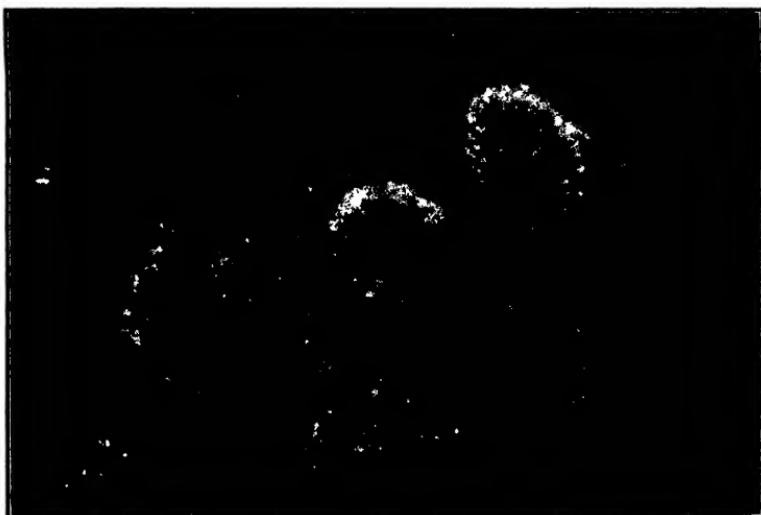


FIG. 1. Telia of *Dicheirinia Ormosiae* showing the dense, encircling, botryoid paraphyses typical of the species. For an illustration of a single paraphysis see plate 16, figure 4. The uredia are identical in gross appearance.  $\times 30$ .

regular or cubical bead-like warts, the pore one, very obscure but apparently apical and next the inner angle; pedicel bearing one apical cell, rarely two cells by a vertical septum, colorless, fragile, breaking below the apical cell or at the spore.

HOSTS AND DISTRIBUTION: *Ormosia Krugii* Urban, in Porto Rico and Santo Domingo.

TYPE LOCALITY: El Yunque, Porto Rico, on *Ormosia Krugii*.

EXSICCATI: R. Cifferi, Mycoflora Domingensis 34.

The fragility and early disjunction of the pedicel probably account for the description of this species under the genus *Puccinia*. Details can be studied satisfactorily only in sections when

the proper orientation of the teliospores is obtained. Occasional association of three spores on a pedicel occurs. The apical cell of the pedicel is then usually and perhaps always divided once, the third spore being borne by one cell, the other two by the second cell.

2. *DICHEIRINIA BINATA* (Berk. & Curt.) Arth. N. Am. Flora 7: 147. 1907.

*Triphragmium binatum* Berk. & Curt. Proc. Am. Acad. 4: 125. 1858.

*Lecytha pesisaeformis* Berk. & Curt. Proc. Am. Acad. 4: 127. 1858.

*Diorchidium binatum* De-Toni, in Sacc. Syll. Fung. 7: 736. 1888.

*Uredo ? pesisaeformis* De-Toni, in Sacc. Syll. Fung. 7: 856. 1888.

*Uredo Cabreriana* Kern & Kellerm. Jour. Myc. 13: 25. 1907.

Pycnia amphigenous and petiolicolous, subcuticular, conical becoming flat and wide spread. Aecia subepidermal, amphigenous and petiolicolous, on hypertrophied areas, large, becoming confluent over extended areas, uredinoid, cinnamon-brown, with few to many discal paraphyses; aeciospores borne singly on pedicels, obovoid-globoid or with one side flattened, 19–26 by 29–35  $\mu$ ; wall 2.5–3  $\mu$  thick or slightly thicker above, sharply echinulate, dark cinnamon-brown, the pores 3 with 1 usually on the flattened side and 2 on the curved side, equatorial, distinct. Uredia subepidermal, mainly hypophyllous, light cinnamon-brown, with numerous discal, branched paraphyses having a thick-walled or solid, refractive, irregular knob-like head (PLATE 16, FIG. 3); urediospores like the aeciospores but more globoid, 22–29 by 28–32  $\mu$ , with wall thicker, 3–4  $\mu$ , and light cinnamon-brown. Telia subepidermal, chestnut-brown, with paraphyses as in the uredia; teliospores (PLATE 16, FIG. 1, 2) in pairs on a common pedicel, obovoid or nearly globoid, flattened on the inner side, 26–29  $\mu$  wide by 35–40  $\mu$  high; wall chestnut-brown, 2–3  $\mu$  thick at sides, thicker above with digitate projections, more abundant above, few or none at the base, the pore one, near the inner angle; pedicel hyaline, with two apical cells, usually breaking below the apical cells.

HOSTS AND DISTRIBUTION: *Erythrina glauca* Willd., in Porto Rico, Cuba, Central America and South America; *E. Crista-*

*galli* L., in Porto Rico; *E. umbrosa* H. B. K., in British West Indies; ? *E.* sp. in Nicaragua.

TYPE LOCALITY: Nicaragua, on unknown host (? *Erythrina*).

ILLUSTRATIONS: Dietel, Ann. Myc. 24: 130. 1924, fig. 1; Dietel, in E. & P. Nat. Pfl. 2nd Aufl. 6: 68. 1928, fig. 52, C.

An apparently common species usually collected only in the urediosporic stage which is characterized by the shape of the urediospores and the clove-like paraphyses. The teliospores are the largest and most coarsely sculptured of any in the genus.

3. **DICHEIRINIA SUPERBA** Jacks. & Holw. Mycologia 23: 333. 1931.

Pycnia subcuticular, amphigenous or caulicolous. Aecia and uredia wanting. Telia amphigenous, caulicolous or petiolicolous, small or becoming confluent, on hypertrophied areas, chestnut-brown, pulverulent, paraphyses few, discal or peripheral, cylindric, thin-walled; teliospores (PLATE 16, FIG. 9, 10) borne two (rarely 3) on a pedicel, oblong or ellipsoid, 12–16  $\mu$  wide by 22–28  $\mu$  high; wall 1–1.5  $\mu$  thick, cinnamon-brown, verrucose with cubical projections, more abundant above, pore one, apical, at the inner angle; pedicel hyaline, with two apical cells, fragile and breaking below the apical cells.

HOSTS AND DISTRIBUTION: *Inga* sp., in Brazil.

TYPE LOCALITY: Petropolis, Rio de Janeiro, Brazil, on *Inga* sp.

ILLUSTRATIONS: Jackson, in Mycologia 23: 334. 1931, fig. 1.

EXSICCATA: Reliquiae Holwayanae 281.

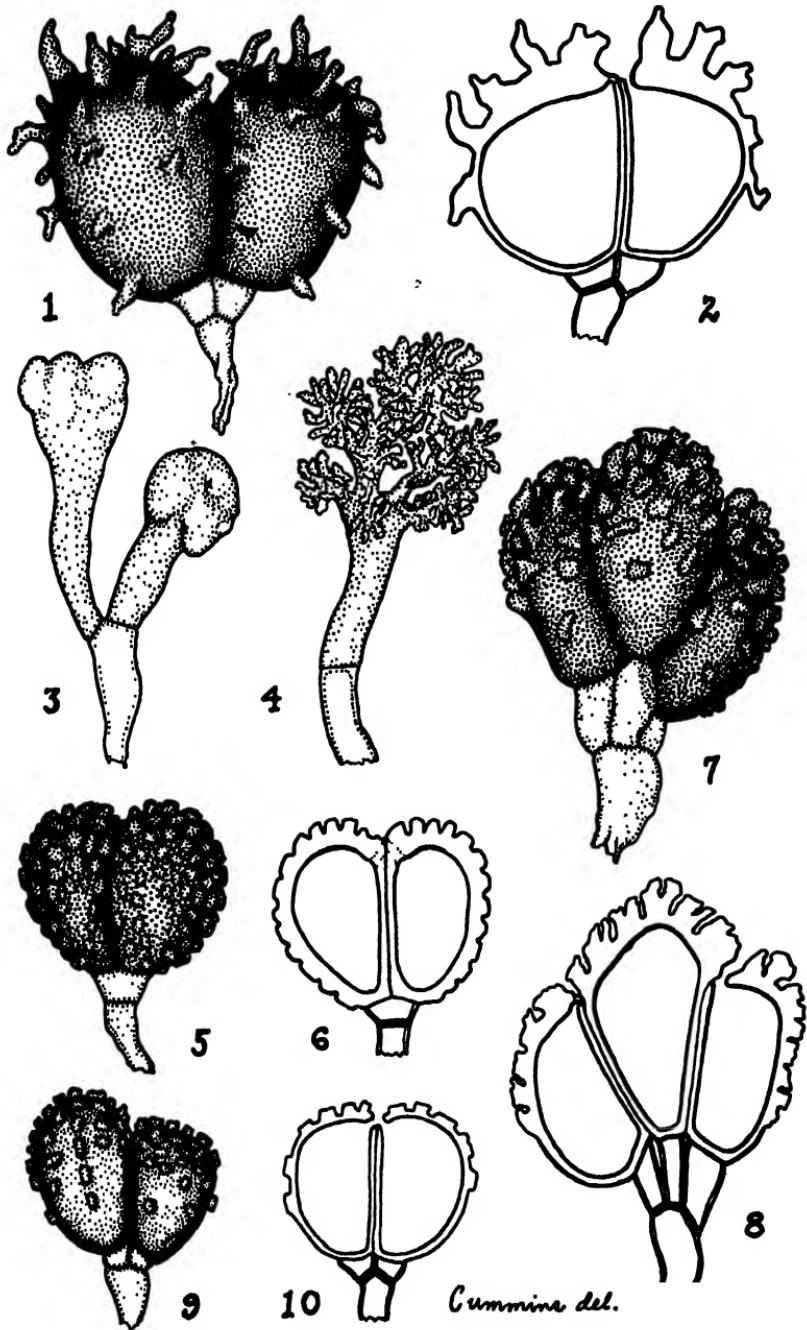
Rarely three spores can be found borne by one pedicel.

As published by Jackson the generic name reads *Dichaerina* but is here corrected to accord with Arthur's spelling, *Dicheirinia*.

4. **Dicheirinia manaosensis** (P. Henn.) comb nov.

*Diorchidium manaosensis* P. Henn. Hedwigia 43: 159. 1904.

Pycnia subcuticular. Aecia and uredia wanting. Telia subepidermal, deep seated, without paraphyses, chestnut-brown, borne on hypertrophied spots on the leaves and stems; teliospores (PLATE 16, FIG. 7, 8) obovoid, 15–20  $\mu$  wide by 25–33  $\mu$  high, borne 3 (rarely 2) on a pedicel; wall 1.5–2  $\mu$  thick, chestnut-brown, coarsely verrucose with cubical or digitate projections, more abundant above, often in rows on the sides and wanting below, pore one, apical and near the inner angle; pedicel hyaline, with three apical cells, short and fragile.



SPECIES OF DICHEIRINIA



HOSTS AND DISTRIBUTION: *Lonchocarpus rariflorus* Mart., in Brazil.

TYPE LOCALITY: Rio Negro, Manáos, Brazil on *Lonchocarpus rariflorus* Mart.

A microcyclic species characterized by its teliospore pedicel with three apical cells each bearing a teliospore. Hennings (*l. c.*) was in error in describing the species as having 2-celled teliospores and made no mention of the structure of the pedicel.

While one spore is commonly somewhat higher than the other two it has its own pedicellate cell and cannot be considered comparable to *Hapalophragmum*. This addition to the 2-spored condition typical of *Dicheirinia* may indicate a tendency toward spore-heads as in *Ravenelia*. *Ravenelia simplex* Diet. offers a close parallel in development.

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#### EXPLANATION OF PLATE 16

All figures were drawn with the aid of a camera-lucida and represent an enlargement of approximately 625 diameters.

Fig. 1, *D. binata*, a perspective drawing of the teliospores to show the peculiar sculpturing of the wall and the structure of the pedicel; 2, *D. binata*, an optical section of the teliospores giving wall thickness and pore location; 3, *D. binata*, refractive, clove-like paraphyses found in the aecia, uredia and telia; 4, *D. Ormosiae*, a botryoid paraphysis as seen in the uredia and telia; 5, *D. Ormosiae*, perspective drawing of the teliospores showing the cubical wall-sculpture and the single apical cell of the pedicel; 6, *D. Ormosiae*, optical section of the teliospores indicating wall thickness and probable location of pores; 7, *D. manaosensis*, perspective drawing of the teliospores to show spore sculpture and pedicel; 8, *D. manaosensis*, optical section of the teliospores; 9, *D. superba*, perspective drawing of the teliospores showing spore sculpture and the two apical cells of the pedicel; 10, *D. superba*, optical section of the teliospores to show the thin wall and location of the pores.

# NEW OR LITTLE KNOWN CHYTRIDIALES<sup>1</sup>

JOHN N. COUCH

(WITH 64 TEXT FIGURES)

During the springs of 1923, 1924, and 1925 a fungus was observed to be parasitic within the threads of *Pythium gracile* and *P. dictyosporum*, the two species of *Pythium* being in turn parasitic in the threads of *Spirogyra areolata* and *Spirogyra* sp. In the development and structure of the plant body the fungus showed a striking resemblance to certain species of *Olpidiopsis*; in the development and behavior of the spores it resembled *Aphanomyopsis* Scherffel and certain species of *Ectrogella* (sense of Scherffel), while in sexual reproduction there was a resemblance to *Pythium*. This peculiar combination of characters separated this fungus from any other previously described and therefore it seemed logical to erect a new genus combining some of the characters of each of the following genera: *Olpidiopsis*, *Ectrogella*, *Aphanomyopsis*, and *Pythium*.

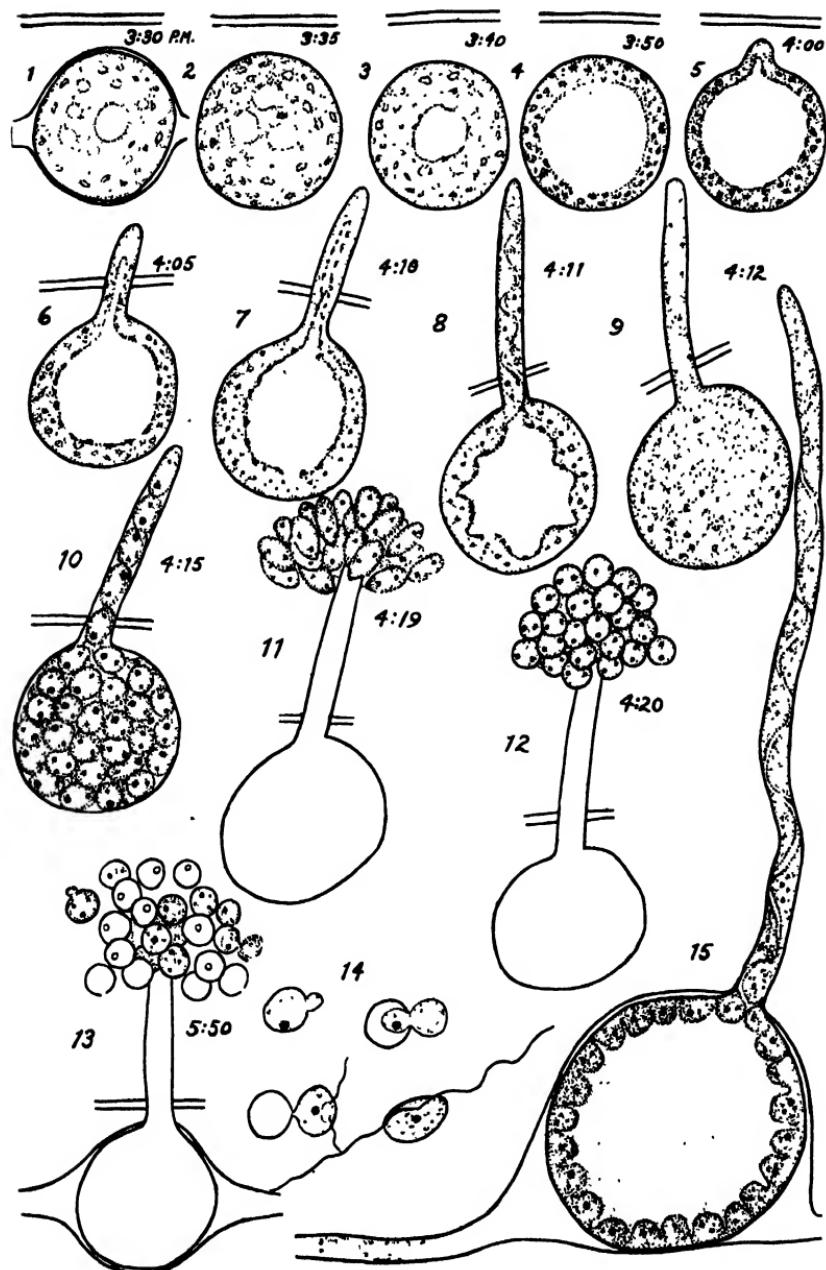
## *Pythiella* gen. nov.

Plant body parasitic within the threads of *Pythium*; without rhizoids, the entire thallus upon maturity being transformed into reproductive organs. Spore development as in the higher water fungi (*Achlya* and *Saprolegnia*, e.g.). Spores after emergence encysting at the tip of the sporangium as in *Achlya*, swarming later in the laterally biciliate condition. Antheridia present on all oögonia. Egg not completely filling the oögonium, and with a distinct periplasm.

### *Pythiella vernalis* sp. nov. (TEXT-FIGURES 1-27).

Sporangia developing in the threads of *Pythium*, spherical or rarely subspherical when mature, without mycelium and rhizoids; causing the formation of a distinct gall in the *Pythium* thread, usually one sporangium in each gall though 2, 3, or 4 may not

<sup>1</sup> Presented before the Mycological Society of America, 1932.

FIGS. 1-15. *Pythiella vernalis*.

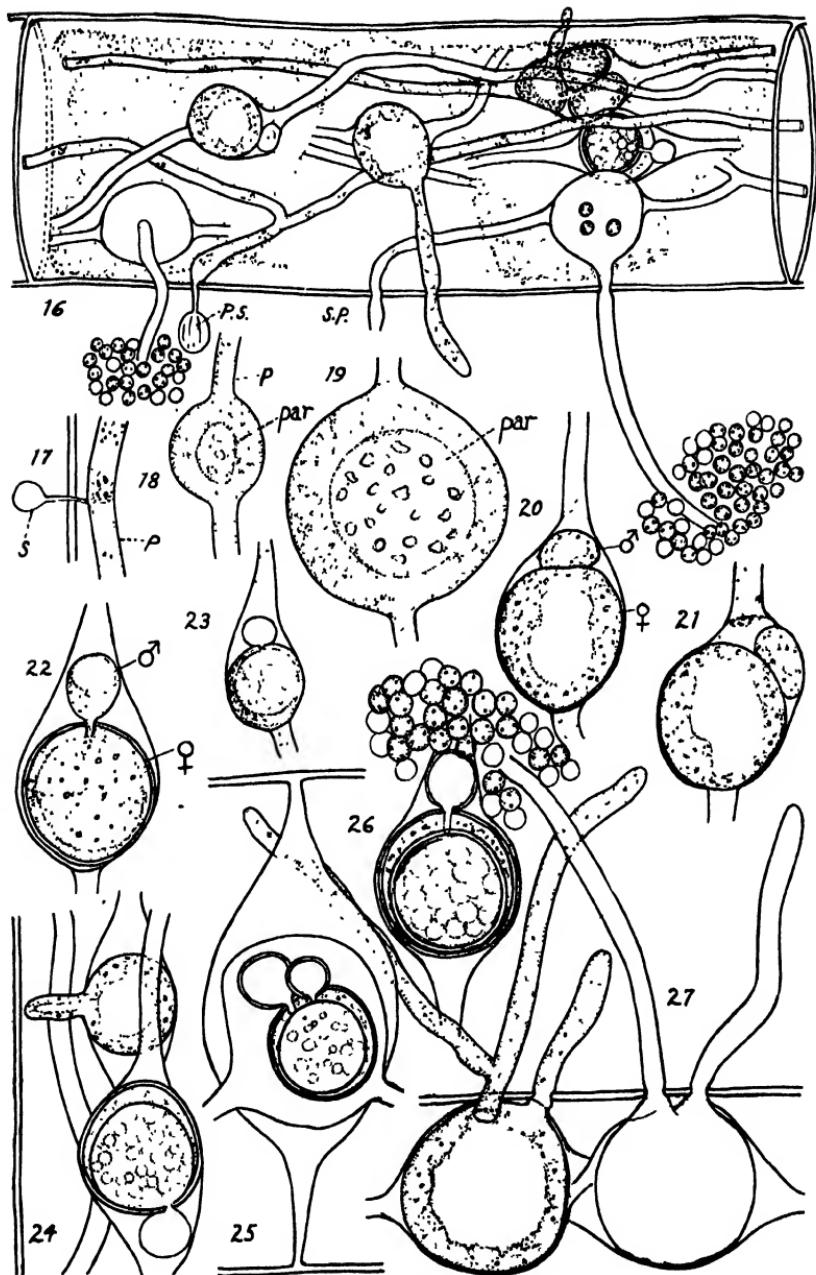
uncommonly occur; 10–30  $\mu$  thick, emptying through a long tube up to 50  $\mu$  long and about 4  $\mu$  thick; on some sporangia several tubes may be formed (as many as five), some of which may be branched. Spores diplanetic, encysting, after discharge, at the tip of the sporangial tube, emerging from the cysts after about an hour, elongated with a longitudinal groove and with two cilia; spores 3.7–4  $\mu$  thick. In swimming the active cilium is directed forward while the posterior one is dragged along behind. Sexual reproduction is by oögonia and antheridia; oögonia 11–18.5  $\mu$  thick, spherical, containing one egg, which does not completely fill the oögonium; eggs 9–15  $\mu$  thick, spherical, when mature surrounded by a thick wall and a small amount of periplasm; antheridium spherical, about 5  $\mu$  thick, emptying its entire contents through a delicate tube into the egg.

Collected several times during the late spring, 1923, 1924, and 1925. Type represented by preserved material on slides in the University of North Carolina Herbarium. Growing as an endophytic parasite within the threads of *Pythium gracile* and *P. dicthyosporum*, the two species of *Pythium* being in turn parasitic in the threads of *Spirogyra areolata* and *Spirogyra* sp.

#### DEVELOPMENT OF SPORANGIA

The spore comes to rest on or near the *Spirogyra* thread, sprouts a fine tube which first penetrates the *Spirogyra* wall and then the *Pythium* thread to discharge its contents into the latter. The empty spore-cyst remains visible on the *Spirogyra* thread for some time after infection. Within the *Pythium* thread, the parasite assumes a rounded or oval shape. It appears to be surrounded by a definite wall and its protoplasm contains numerous, large, glistening granules which are considerably larger than the granules in the *Pythium* thread. As the parasite develops, the host becomes swollen in the region of the thread immediately around the parasite, forming a gall much like that formed in the Saprolegniaceae by *Olpidiopsis*. As a rule each gall contains only one sporangium but I have seen several which contained 2, 3, or sometimes even 4 sporangia, and in such cases the sporangia are more or less flattened by mutual pressure.

It is sometimes quite difficult to determine whether the parasite is within the *Pythium* thread or is merely loose in the *Spirogyra* cell. This difficulty is increased where the gall is partly obscured by the disintegrating contents of the *Spirogyra* cell. Fortunately

FIGS. 16-27. *Pythiella vernalis*.

the material was very abundant and by finding places where the contents of the *Spirogyra* cell had mostly disintegrated it was possible to determine beyond doubt that the galls are within the *Pythium*. A number of cases were seen where the *Pythium* thread, still connected with the old, infecting spore-cyst, contained several of the parasites. Other cases were seen where a thread connected with a sporangium contained one of the parasitic bodies. Again several places such as that shown in figure 25 were seen. Here the gall containing an oögonium and two antheridia has developed in the *Pythium* thread between the two parts of the cross wall of the *Spirogyra* cell. Sometimes the *Pythium* threads may extend from one *Spirogyra* thread through the water to another thread. I have never observed such threads attacked by the parasite. However, I have observed numerous sporangia and sexual bodies of the parasite lying in trash in the water apparently unconnected with the threads of the *Pythium* or *Spirogyra*. If one looks closely, however, he may detect the disintegrated remains of the *Pythium* and *Spirogyra* threads.

Unfortunately no satisfactory tests were made to determine the chemical composition of the cell wall of the parasite while the material was fresh. Some of the material preserved on a slide in glycerine in 1925 was washed and tested (May 1933) with chlorzinc iodide. The *Spirogyra* and *Pythium* walls, where the latter were exposed, gave a beautiful blue reaction but the parasites in the *Pythium* were uncolored by the reagent even where the tubes were projecting out through the *Pythium* and *Spirogyra* walls. This test was performed on two lots of material with results as indicated above and, while not entirely convincing, it suggests strongly that the wall membrane of the parasite is not pure cellulose but rather has a composition similar to that of the Chytridiales.

Shortly before the sporangium attains its mature size the large granules disappear and the protoplasm, except for several small vacuoles, becomes homogeneous. After the sporangium becomes mature in size the development of the spores proceeds rapidly. The small vacuoles flow together to form a single, large, central one. The pressure within this vacuole appears to increase so that the protoplasmic layer becomes quite thin. Meanwhile the tube for the emergence of the spores is formed. As this tube grows

the vacuole extends out through its center. The protoplasm, both in the sporangium and in the tube, becomes arranged in numerous irregular heaps as the furrows of the vacuole push outward toward the cell wall. This stage corresponds to the "spore initial" stage in the Saprolegniaceae and the "balling" stage in *Ectrogella* and *Aphanomyopsis*.

The vacuole pushes outward until suddenly it breaks at one or more places and almost immediately the protoplasm appears to occupy the entire space in the sporangium. This change appears to be accompanied by a decrease in the size of the sporangium. This stage corresponds to the "homogeneous" stage in the development of the sporangium in the Saprolegniaceae.

In a very few minutes (3-5) after the "homogeneous" stage, the completely formed spores appear. The spores are not equipped with cilia and appear motionless within the sporangium. The pressure within the sporangium increases so that the tip gives way and suddenly the spores rush out with great rapidity. Usually all the spores emerge but sometimes a number may be left in the sporangium to encyst there. Immediately after the spores emerge, they are elliptic and somewhat pointed at the bases which seem to be connected with the tip of the emergence tube. The spores retain this shape for only a few seconds, quickly rounding up and encysting in the same position. Each spore possesses two or more conspicuous granules.

After remaining quiet at the sporangial tip for some time (usually one to two hours) the spores emerge from their cysts. This process is as in *Achlya* or *Saprolegnia*. The zoospore is elongated, ovoid, with a median groove from which arise two cilia. As the spore swims the active cilium is directed forward while the posterior cilium is dragged along behind. The movements of the spores through the water are smooth, the spore describing a spiral path as it moves forward and also rotating on its axis, the movement being like that described by Weston (1918) for the zoospores of *Thraustotheca clavata*.

#### DEVELOPMENT OF THE OÖGONIA AND ANTERIDIA

Although I have observed a large number of stages in the development of the sexual organs of this fungus, I have not followed

through their development. Each oögonium is accompanied by a much smaller antheridial cell and not rarely there may be two of the male cells applied to one oögonium. Both cells are contained within the host thread. After the oögonium reaches its mature size one may recognize a large irregular vacuole. This increases in size, the parietal protoplasm being simultaneously thinned down in a few places and heaped up in others. The number of "heaped-up masses" in the oögonium at this time is many fewer than in the sporangium when the spore origins appear. I have not followed the ultimate fate of the vacuole but it continues to push outward, the parietal protoplasm becoming thinner and thinner at certain places. At a later stage the contents of the oögonium are divided into a dense central spherical mass and a thinner peripheral layer of protoplasm. Although the nuclear details have not been worked out, it appears that the inner spherical mass is an egg and the outer layer is periplasm. The antheridium sends a delicate tube through the oögonial membrane and periplasm into the egg and into this discharges its entire contents. As the egg matures, its wall becomes considerably thickened and all or nearly all of the periplasm disappears. Presumably this takes part in the thickening of the egg wall. I have not observed the germination of the eggs.

#### RELATIONSHIPS

This fungus is apparently very closely related to *Ectrogella* in the sense of Scherffel and to *Aphanomyopsis* Scherffel. The genus *Ectrogella* was established by Zopf (1884) on the single species *E. bacillariacearum* but Scherffel (1925) has added several new species and has brought to light new information which indicates a close relationship between this genus and the Acanthocystineae and Saprolegniaceae-Peronosporaceae series rather than to the Chytridiaceae series. In *Ectrogella* the sporangia have a central vacuole. A "balling stage" occurs. The zoospores usually have two cilia and swim smoothly. These characters belong to the former series rather than to the Chytridiaceae. Scherffel was the first to call attention to the difference in the appearance of the protoplasm in the vegetative threads of the Saprolegniaceae and the cells of the Chytridiales and the Acanthocystales, the protoplasm in the former having a granular consistency while in the

latter two orders it has a pale whitish fat-gleam. This difference is striking and can easily be observed in *Myzocytium* and *Lagenidium* (probably also in *Ancylistes*, though I have not noticed it). The protoplasm of *Ectrogella* and *Aphanomyopsis* is like that in the Saprolegniaceae and therefore Scherffel thinks them to be more closely related to the latter order than to the Ancystiales.

While the present species is close to certain species of *Ectrogella* (*E. monostoma*) and *Aphanomyopsis* in spore development and behavior, it differs from them both in the appearance of the protoplasm, for here it resembles that of *Myzocytium*, *Lagenidium*, and *Olpidiopsis* in having a pale, whitish gleam and in containing a few, large, conspicuous, irregular, glistening granules. Moreover, the spores of *Aphanomyopsis* are almost twice as large as in the present species.

In sexual reproduction the present species is distinct from *Aphanomyopsis* and *Ectrogella*. In *Aphanomyopsis*, according to Scherffel (1925), the resting spores are like the oöspores of *Saprolegnia* in structure, but arise asexually in a rudimentary oögonium. In one species of *Ectrogella*, *E. Licmophorae*, resting spores are formed and these arise by a sexual act, apparently much as in *Myzocytium proliferum* (Scherffel, 1925).

The present species may easily be distinguished from *Myzocytium*, *Lagenidium*, and *Achlyogeton*, to each of which, however, it shows certain similarities in structure which perhaps indicate homoplasmy rather than genetical relationship. It may also be distinguished from *Achlyella*, an imperfectly known genus described by Lagerheim (1889), by the extramatrical sporangia of the latter. The two genera agree in the encystment of the spores at the sporangial tip. In *Achlyella*, however, the size, shape, number of cilia, and method of swimming of the spores are not known, and therefore a satisfactory comparison of these two genera must wait until *Achlyella* can again be found and adequately described. It also resembles *Olpidiopsis Schenkiana* Zopf in sexual reproduction, but may be distinguished from that species by spore behavior and the absence of periplasm in the oögonia of *O. Schenkiana*. Furthermore, the chemical composition of the cell wall in *Olpidiopsis*, which gives a beautiful purplish reaction with chlor-zinc iodide, would exclude the present species from that genus.

This species shows a superficial resemblance to *Pseudolpidium Aphanomyces* (Cornu) Fischer as described and illustrated by Butler (1907). The galls, the shape of the sporangia, and their emergence tubes are quite similar, but the spore behavior in the two genera is markedly different.

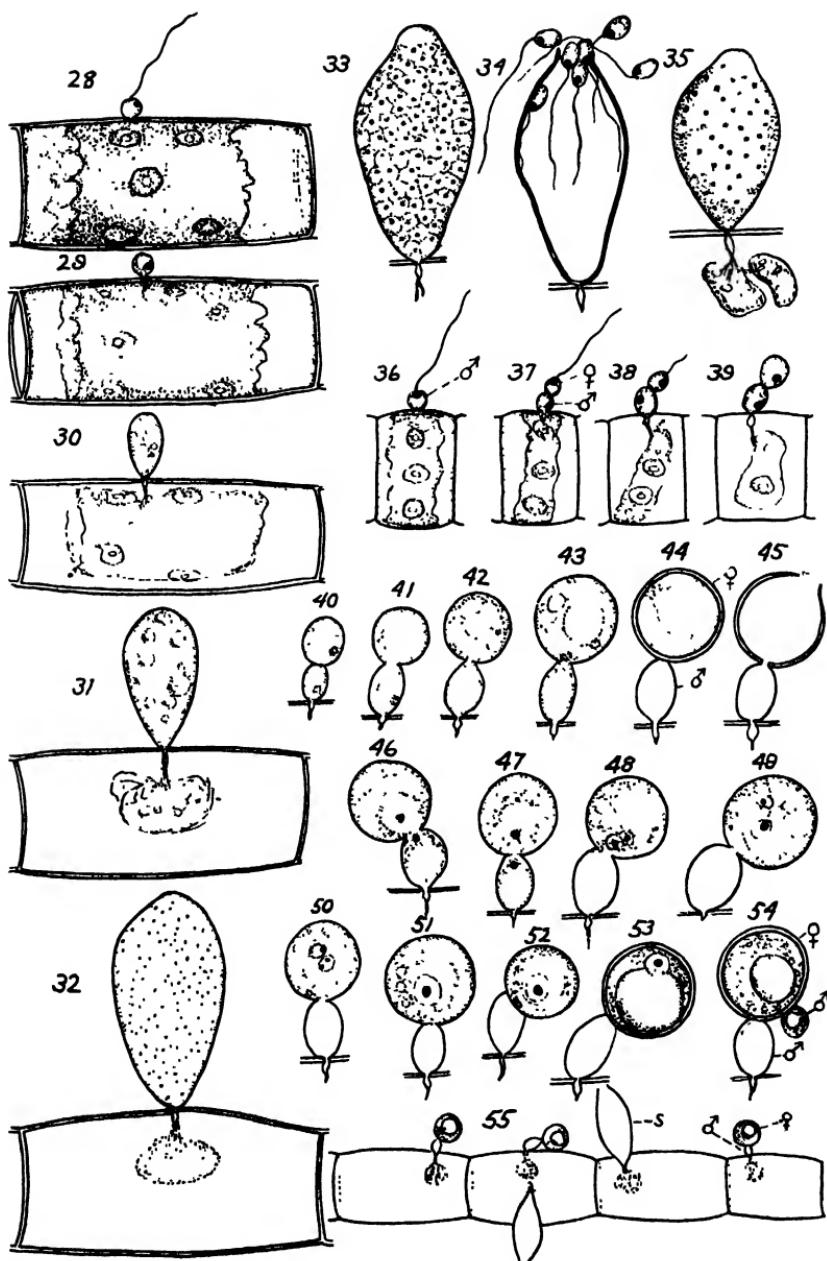
The following species of *Rhizophidium* has appeared only once. Its peculiar type of resting spore formation, in which the female gamete is the more active and searches out the male, perhaps justifies its presentation here.

***Rhizophidium ovatum* sp. nov. (TEXT-FIGURES 28-55).**

Mature sporangia pear-shaped or oval, thickest in the distal half; attached to the host by a minute bulbous swelling from which arises a very slender rhizoid which extends to the chromatophore of the host. Sporangia  $8.4-16.8 \times 16-30 \mu$ , most about  $13 \times 20-25 \mu$ . Spore formation as in the genus. Sporangial dehiscence apical. The tip of the sporangium gelatinizes, suddenly bursts and the spores emerge with great rapidity, the sporangium becoming empty in a few seconds. Spores swimming away upon emerging, slightly elongated, about  $3 \times 4 \mu$ , with a large oil globule and a long posterior cilium. Spores swimming by a darting and hopping motion. Sexual reproduction of a peculiar type: the male cell settling down on the host, penetrating the latter to develop the bulbous swelling and minute rhizoid; the female cell later attaches itself to the male. Now fed indirectly from the host through the male, the female increases rapidly in size; the male cell increasing in size considerably or not at all, but finally emptying its entire contents into the female. Male cells or antheridia usually spherical or slightly subspherical,  $3.6-5 \mu$ , most about  $4.4 \mu$  thick; often oval and then usually larger, such antheridia being up to  $5 \times 7 \mu$ ; female cells, or oögonia, when mature,  $5.4-9.6 \mu$ , most about  $8.4 \mu$  thick, spherical, when ripe with a slightly thickened wall and a single, large, slightly excentric oil globule. Oögonia germinating after a rest period of two or three days to form spores.

Found only once, May 6, 1931, on *Stigeoclonium* (?) sp. in small wet weather pond on way to Laurel Hill, Chapel Hill, N. C.

This species apparently belongs to the genus *Rhizophidium*, though because of the minute bulbous base and the very fine rhizoid which is very difficult to see one might put the species in the genus *Phlyctidium*. In fact the rhizoid was so fine that I could

FIGS. 28-55. *Rhisopodium ovatum*.

never be absolutely certain if there were only one rhizoid or several.

In asexual reproduction the present species is close to *R. Fusus* (Zopf) Fischer and *R. Lagenula* (A. Br.) Fischer. In *R. Fusus* a very extensive rhizoidal system is developed, while in the present species the rhizoid or rhizoids are very minute. Both fungi have elongated sporangia but in *R. Fusus* the sporangia are thickest in the middle while in *R. ovatum* they are thickest in the distal half. In *R. Fusus* the resting spores have not been observed. *Rhisophilidium Lagenula* may be easily separated from *R. ovatum* by the fact that the former is slightly longer and much thinner than the latter.

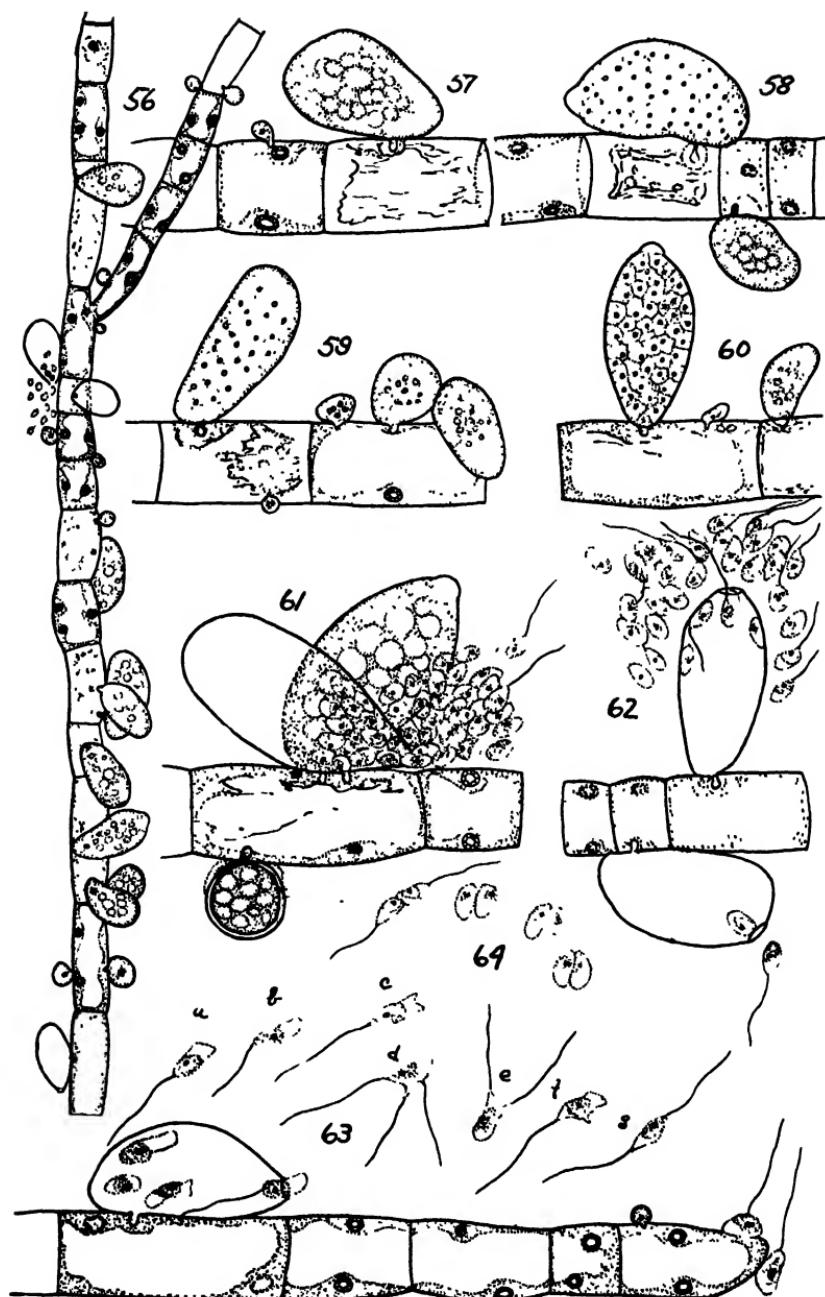
In sexual reproduction the fungus appears to resemble *R. granulosporum* Scherffel. In this species Scherffel (1925b) shows the mature zygote attached to the smaller, empty, male gamete rather than to the host cell. The male gamete is attached to the host cell as in the present species. The wall of the zygote in *R. granulosporum* Scherffel is spiny while in *R. ovatum* it is smooth. Moreover, the shape of the sporangia in the two species is quite distinct.

I am unable to say whether the gametes are borne in the sporangia along with the zoospores or are formed in separate gametangia.

Fixed material stained with Gram's gentian violet showed that the gametes are uninucleate and that the nucleus from the smaller, male cell passes along with the cytoplasm over into the female cell, fusing with the nucleus of the latter.

**Phlyctidium anatropum** (Braun) Sparrow (TEXT-FIGURES 56-64).

Sporangia attached to the filaments of *Stigeoclonium* by means of a small rounded haustorium; very irregular in shape, usually ovoid and flattened on one side, often attached on the flattened side, sometimes ovoid and symmetrical;  $9-14 \times 16-25 \mu$ . Spore development somewhat as in *Rhisophilidium*. As the sporangium matures a hyaline papilla is formed on one end of the sporangium. When the spores are mature, the sporangial wall gives way at this point and the spores emerge. Spores about  $2.1 \times 5 \mu$ ; encysted spores subglobose, about  $2.2-3 \mu$  thick, with a small, inconspicuous dot or fat globule. The first spores emerge more rapidly than the later ones though they all come out with characteristic slowness. As a rule they hover around the sporangium for some time



Figs. 56-64. *Phlyctidium anatropum*.

after emerging. Spores uniciliate, but the cilium is apparently functionless since the spores move in an amoeboid fashion dragging the cilium along behind. Spores frequently forming one or several hair-like pseudopodia. Uniciliate gametes are formed which unite in pairs, perhaps fusing, the zygote apparently developing into a thick-walled, spherical, resting body about  $10\ \mu$  in diameter.

Easily recognized by the asymmetrical sporangia and by the uniciliate, amoeboid zoospores.

On *Stigeoclonium* sp., Chapel Hill, N. C., May 15, 1931; also collected by Sparrow on *Stigeoclonium* sp., Bessemer, N. Y., January 1932.

When this organism was first seen by me, I took it for encysted stages in some peculiar protozoan. It was not until its development was followed and sporangia in the act of spore discharge were observed that its true nature was suspected. The sporangia have a rather unique appearance in the stages preceding the beginning of spore formation. This is not shown sufficiently distinctly in any of my figures except perhaps the larger sporangium in figure 57 and the smaller one in figure 58. There is a thick, clear, outer region of protoplasm enveloping the inner region of fat globules. When the time for spore formation approaches, the globules of fat (?) are digested and numerous minute globules are evenly distributed throughout the protoplasm (FIG. 58 AND 59). As the spores are formed, each one contains a single, small globule but these bodies are not so conspicuous as in the spores of *Rhizophidium*.

By isolating material early in the morning and making frequent observations throughout the day, it was found that the spores are usually discharged between 6 and 8 o'clock in the evening. Spore discharge was observed about the same time on several successive evenings, occurring about the same time in the original material in a Petri dish as in material isolated on slides. The first spores to be discharged and by far the greater number are apparently forced out by some internal pressure, the cilium being dragged behind as they pass through the opening. The later spores creep out by amoeboid motion. Due to the inactivity of the cilia the spores remain for several minutes in a loose cluster at the tip of the sporangium and then slowly creep away. The spores creep over the surface of the threads and over the slides, the movement

keeping up for over an hour but in no instance have I observed any other motion than the amoeboid type. The spores are elongate and are composed of two distinct regions: a region of granular protoplasm to which the cilium is attached and a hyaline zone, which makes up about half the spore, opposite the ciliary end. It is this hyaline zone which changes shape, producing at times short pseudopodia (FIG. 63c) or at other times long hair-like ones (FIG. 63d).

Spores or gametes were frequently observed grouped in pairs but I could not be positive about their final fusion (FIG. 64). My observations were quite similar to those of Ledingham on a species of *Rhizophidium* (in paper given before the Mycological Society of America, Christmas meeting, 1932), who not only observed the fusion of the gametes but also observed that the zygote formed resting sporangia. After the Christmas meetings, I looked over my slides again and found a considerable number of thick-walled resting sporangia attached to the host threads with similar, minute, bulbous haustoria as found on the zoösporangia.

The present fungus appears to be identical with Sparrow's (1933) description and figures of *Phlyctidium anatropum* (Braun) Sparrow. Sparrow's fungus, like mine, occurred on *Stigeoclonium* sp. and agrees in nearly all details. He did not observe spore discharge, in fact, if the species is correctly identified, this is the first time spore discharge has been observed. I have not seen Braun's original paper but, according to Sparrow, Braun observed thick-walled ovoid resting sporangia. In the genus *Phlyctidium*, several species of which I have studied, the spores are spherical with a single large globule, and uniciliate, the movement of the spores being characteristically of the chytridiaceous type and not amoeboid. The peculiar amoeboid movement of the spores with the formation of the hair-like pseudopodia would seem to separate the present species rather sharply from the genus *Phlyctidium*. Since the synonymy of this species is already rather complicated it would be better to wait until its complete life history is certainly known before removing it from *Phlyctidium*.

The damage done to the algal host is not so noticeable in fresh material as in material preserved in glycerine. However, as a rule, even in fresh material one can see that the protoplasts of the host are disorganized as the parasite develops. In preserved material

the injury is more noticeable, due to the greater plasmolysis of the parasitized cells.

#### SUMMARY

A new genus containing one species (*Pythiella vernalis*) is described which is related to *Ectrogella*, *Aphanomyopsis*, and *Olpidiopsis Schenkiana*. The fungus is parasitic in the threads of *Pythium gracile* and *P. dictyosporum* which are in turn parasitic on the threads of *Spirogyra arcolata* and *Spirogyra* sp. In asexual reproduction the fungus resembles *Ectrogella* while the presence of periplasm in the oögonium suggests a remote relationship to *Pythium*. The protoplasm of *Pythiella* has the pale whitish gleam and the fat globules characteristic of the Chytridiales and Acanthistales whereas in *Ectrogella* the protoplasm has a granular appearance as in *Saprolegnia*. It would seem that *Pythiella* should be placed in the Chytridiales close to *Olpidiopsis Schenkiana* (sense of Butler and Scherffel).

A new species of *Rhizophidium*, *R. ovatum*, parasitic on *Stigoclonium*, is described. It is characterized by the ovate sporangia, the very minute rhizoids, and the peculiar type of sexual reproduction in which the female gamete is the more active. The gametes are uninucleate. The contents of the male cell pass into the female and the two nuclei unite. The zygote after a short period becomes a zoösporangium.

*Phlyctidium anatropum* (Braun) Sparrow is described. The organism is recognized by the asymmetrical sporangia and by the uniciliate but amoeboid zoöspores. The latter are described for the first time. Uniciliate gametes (?) which unite in pairs, perhaps fusing, to form a thick-walled, spherical, resting body are described.

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#### EXPLANATION OF FIGURES

(Figures inked in by Miss Alma Holland)

Fig. 1-27. *Pythiella vernalis* Couch (1-14, illustrate continuous observations on one single sporangium). 1-4, show coalescence of small vacuoles to form a large central vacuole; 5-7, show formation of emergence tube; 8, appearance of spore origins; 9, disappearance stage; 10, reappearance of spores; 11, spores immediately after discharge and before encystment; 12, spores encysted; 13, spores emerging from cysts one hour and thirty minutes later; 14, more highly magnified view of spores emerging from cysts and showing cilia; 15, section view of large sporangium showing stage just before disappearance of spore origins; 16, habit sketch showing parasites within *Spirogyra* cell. Note disintegrating chloroplasts; *Pythium* threads in which are asexual and sexual stages of *Pythiella*; also empty spore cyst of *Pythium* (p.s.) and empty sporangium of *Pythium* (s.p.); 17, early stage of infection. s, empty cyst of *Pythiella*; p, *Pythium* thread; 18 and 19, development of the gall. p, *Pythium* thread; par, *Pythiella*. Note granules of fatty substance in protoplasm of latter; 20-26, stages in the development of oögonia and antheridia. Note periplasm in figures 22-26. Figure 25 shows parasitic gall formed in *Pythium* thread between end walls of two *Spirogyra* cells; 27, two sporangia with several emptying tubes, one of which is branched; All above figures  $\times 1350$  except figure 16 about  $\times 675$  and figure 14 about  $\times 2000$ .

Fig. 28-55. *Rhizophidium ovatum* Couch. 28-34, stages in development of one single sporangium from infection by spore 7:30 A.M. to 3:27 P.M. the following day. Note disintegration of chloroplast; 35, sporangium a few hours before spore discharge; 36-44, development of zygote, living material; 45, zygote which has formed a sporangium; 46-53, killed and stained material showing development of zygote; 54, zygote with two male cells; 55, habit sketch showing sexual and asexual reproductive bodies. Fig. 28-54  $\times 1250$ ; fig. 55  $\times 620$ .

Fig. 56-64. *Phlyctidium anatropum* (Braun) Sparrow. 56, habit sketch showing numerous parasites on thread of *Stigeoclonium*; 57-60, sporangia in various stages of development. The large sporangium in figure 57 and the smaller one in figure 58 show thick, peripheral, hyaline zone of cytoplasm surrounding central region of globules. Larger sporangia, figure 58 and 59, show minute droplets, one for each spore; 61-63, sporangia with spores in the act of emerging. Note how spores hover around sporangial mouth, figure 61 and 62. In figure 63 the letters show successive changes in the same spore as it was watched during a period of several minutes. In figure 61 one resting sporangium is shown; 64, gametes in act of fusing. Four pairs are shown. Many such were seen. Fig. 56  $\times 620$ ; others  $\times 1250$ .

# SOME NON-CATENULATE CONIDIAL PHY-COMYCETES PREYING ON TERRICO-LOUS AMOEBAE

CHARLES DRECHSLER

(WITH 5 TEXT FIGURES)

In a summary (3) published in 1933 were set forth briefly the morphological features of five fungi that had been found capturing and killing *Amoebae* in aging agar plate cultures started from plantings of diseased rootlets and other decaying vegetable materials. The continuous mycelium in four of the five forms (3, figs. 2-5) obviously characterized them as Phycomycetes, as did also the direct origin of the subspherical sexual spores through fusion of paired filaments figured for three of the species (3, figs. 3-5). No definite assignment of the fungi was then attempted, partly for the reason that the meager differentiation of the fusing elements, together with the extraordinarily small dimensions of the sexual apparatus, introduced serious difficulties of interpretation. Moreover, though the study of the asexual reproductive phase entailed less optical uncertainty, the conidia showed no close parallelism in structure or in manner of origin to those of any of the better known groups in the Phycomycetes, being suggestive rather of types known among the Mucedinaceae especially with respect to shape and, in two of the species, to the presence of empty appendages.

More recently the four minute non-septate predacious forms were discussed (6) as members of a new major group of Phycomycetes for which the term Zoopagaceae was suggested as being a tolerably appropriate one. The fairly unambiguous morphology of the several remarkable endoparasitic, ectoparasitic and predacious species about which more particularly the new group was integrated, furnishes now a background for a more satisfactory description of the minute *Amoeba*-capturing organisms than could have been undertaken earlier. In addition to the four taken up in the summary, an equal number of closely related predacious

species that later came under observation are included for discussion. All eight are represented in their vegetative phase by an extensive mycelium on which terricolous *Amoebae*, mostly of the smaller and medium sizes, are captured through adhesion. The general biological relationship is thus broadly comparable to that described previously in the account of *Zoopage phanera* Drechsl., though the endozoic parts, instead of constituting a distinctive compact haustorium, make up a branching system only little differentiated from the mycelium generally.

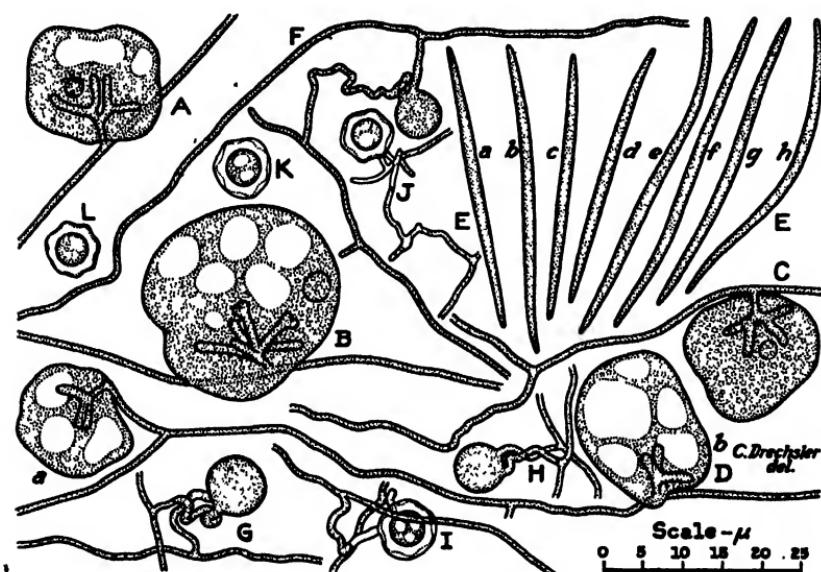


FIG. 1. *Acaulopage rhipidospora*.

If the conidia of the species herein described are hardly to be regarded as of large size in comparison with those of fungi generally, they are yet of generous dimensions when viewed in relation to the delicate mycelia and the minute sexual structures associated with them. An analogous proportionality in size obtains in the predacious *Zoopage phanera*, but not in any of the related parasites, internal or external, assigned (6) to the genera *Endocochlus*, *Cochlonema* and *Bdellospora*, even though the latter two genera share in the catenate development of asexual spores represented in *Zoopage*. The explanation of these size relationships lies very

probably in the requirements entailed in a predacious as contrasted with a parasitic habit. A conidium designed to be ingested by its *Amoeba* host manifestly needs to be no larger than of a size just sufficient to start a new thallus. The production of a short germ tube and of a minute thallus later to be detached, exemplified in the development of the conidium of *Endocochlus asteroides* Drechs., likewise requires only a moderate expenditure of protoplasmic substance. Nor does the proliferation of an incipient haustorium by the conidium of *Bdellospora helicoides* Drechs., preceding the instigation of autonomous development, require any great outlay of material. On the other hand, an organism dependent for its existence on the capture of animals as minute and as slow of movement as the smaller *Amoebae*, and besides present often only in rather small number, would obviously have need from the very beginning of a fairly extensive mycelium. The interception and capture of prey under conditions at all difficult would obviously require beforehand a rathy development of germ hyphae from the substance of the conidium itself (FIG. 5, F).

Because of their dimensions and the frequency with which they make their appearance on old isolation plate cultures, the conidia of the fungi under consideration can hardly have escaped being seen by mycologists at least occasionally in the course of routine operations. That they have evoked little if any comment is very probably to be attributed in part to their mostly commonplace appearance, and in part to the difficulty of determining the manner in which they are borne. Thus, for example, the very distinctive conidia produced by the species now to be described as *Acaulopage tetraceros* have for many years put in appearance from time to time in my own cultures, but their haphazard distribution on the substratum and the absence of anything recognizable as conidiophores, uniformly gave the impression that they represented spores of some *Tetraploa*-like hyphomycetous form that had been introduced accidentally and been scattered about through disturbances incident to microscopic examination. Similarly the sexual apparatus of some of the forms described herein are objects that have been long familiar to me in old isolation plate cultures, but since the hyphae supporting them become almost wholly invisible after being evacuated, they were confused with the angular cysts of

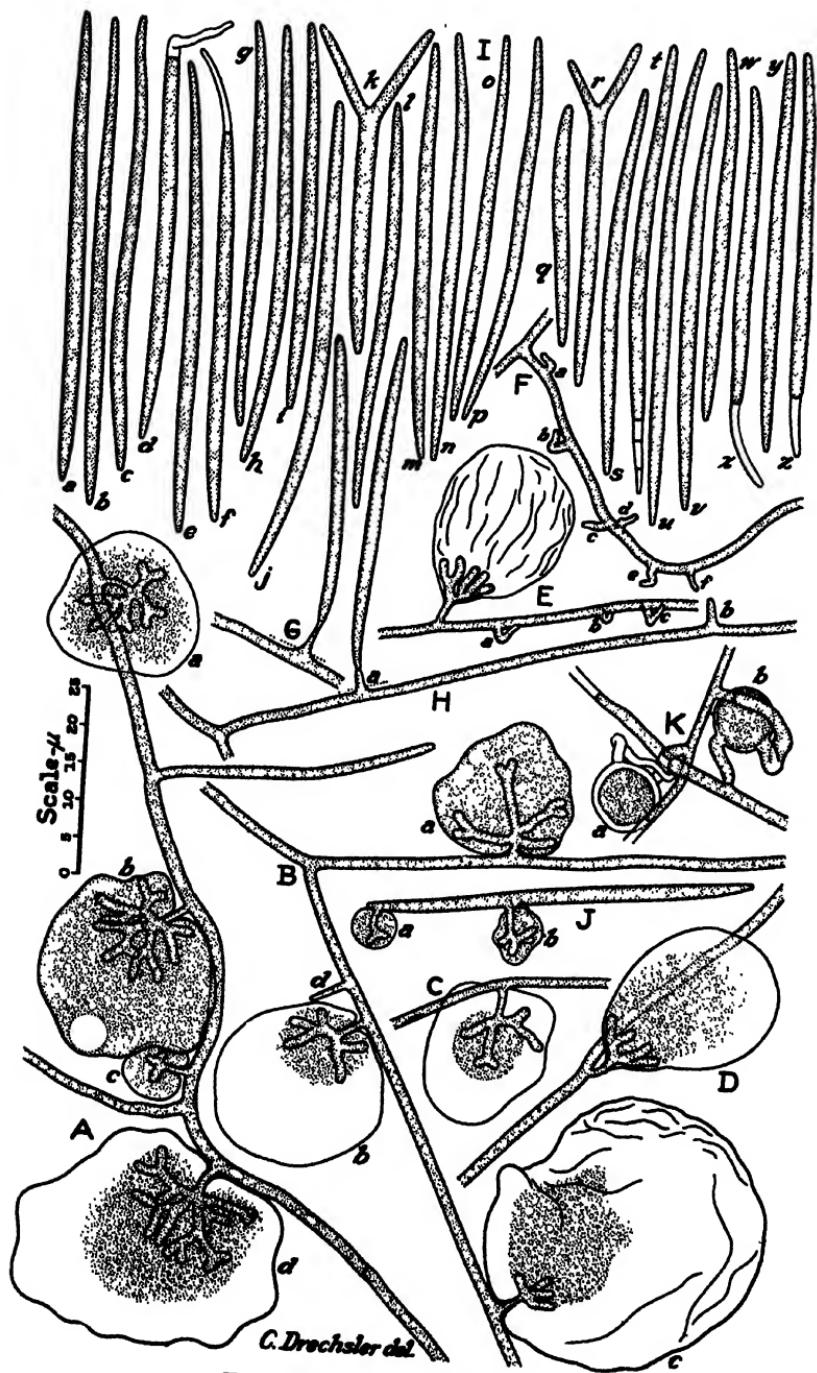


FIG. 2. *Acaulopage macrospora*.

some small protozoans habitually and abundantly present in such preparations.

Undoubtedly, too, the very fact that predacious fungi ordinarily make their appearance in an isolation plate culture only after it has been overrun by various much more luxuriant and conspicuous saprophytic forms, has helped to keep them in obscurity. This tardiness in making their appearance is due evidently not so much to the generally somewhat slow rate of growth of the fungi themselves as to the time required for conditions to arise permitting any development of them at all. Conidial apparatus is usually not produced in such quantity as might invite notice until the underlying vegetative mycelium has attained some extension. Since all members of the group are apparently entirely dependent for their nourishment on animals, extensive mycelial growth of the predacious forms can not take place until prey is present in quantity. However, an abundant supply of *Amoebae*, or for that matter, of nematodes, is usually not available until the relatively few living specimens in the piece of decaying vegetable material from which the culture was started, have multiplied for a week or two. There is evidence indicating that the animals in question feed on the bacterial slime and fungous spores present on the cultures, rather than directly on the agar substratum; so that the establishment first of a congenial fungous and bacterial flora, and then of a suitable fauna of microscopic invertebrates, needs to precede any noticeable development of predacious types. As might be expected under the circumstances, predacious forms unlike many of the common Phycomyces, show no mycelial degeneration from contact with bacterial slime.

The character of the rapidly growing fungi first extending themselves over an agar plate, in influencing the trend of subsequent development of a subsidiary microflora, and thereby the composition of the infesting microfauna, affects greatly the abundance and identity of the predacious Phycomyces appearing later, just as it affects greatly the abundance and identity of the predacious Hyphomycetes. Fungi with a dense dry aerial mycelium, or with sporophores in dense arrangement, as, for example, species of *Mucor*, *Rhizopus*, *Penicillium*, *Alternaria* or *Hormodendron*, not only give little encouragement to bacterial development, but im-

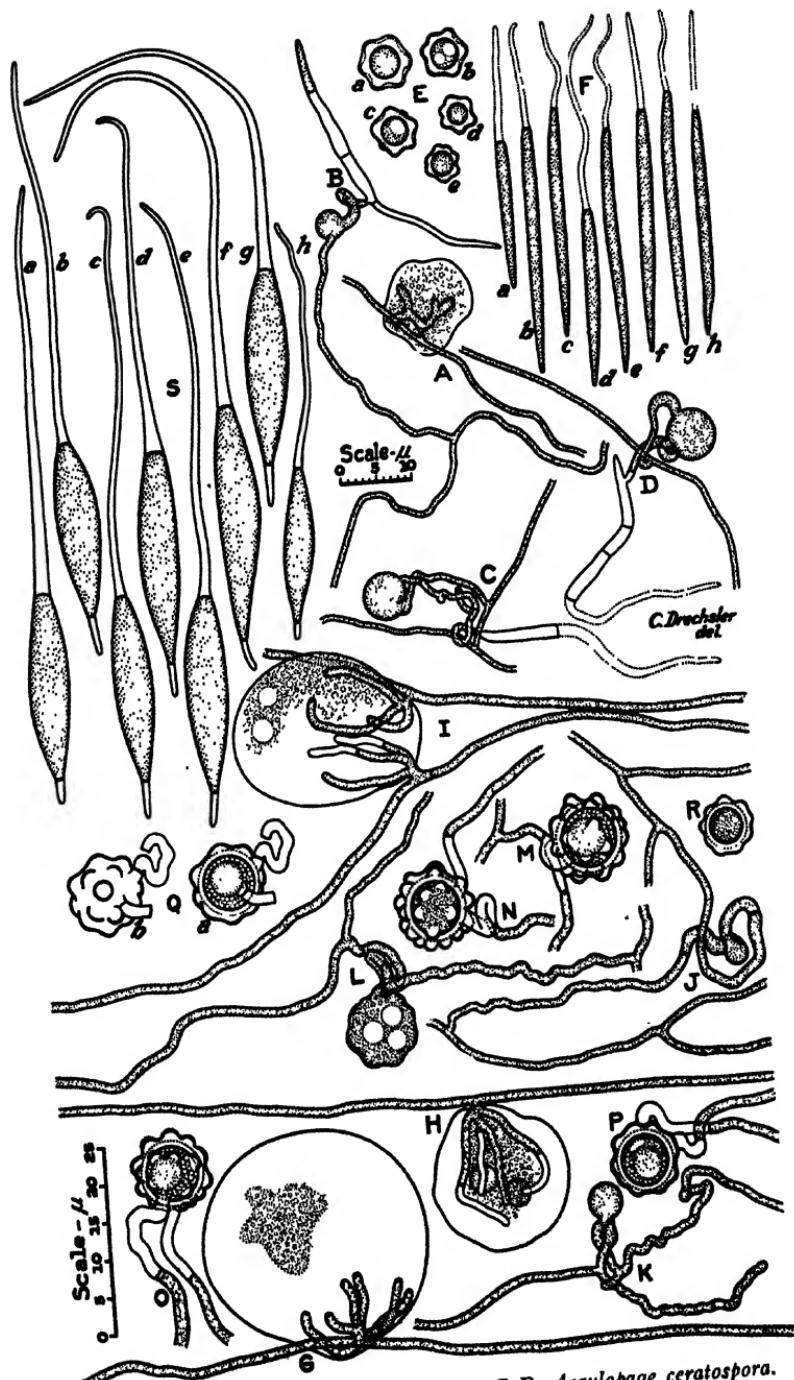


FIG. 3. A-F, *Acaulopage rhinopora*. G-R, *Acaulopage ceratospora*.

pede physically the locomotion entailed in the feeding activities of *Amoebae* and nematodes. If fungi of such character are first to establish themselves in a Petri dish culture, prospects for the development of predacious forms like those herein discussed, are decidedly poor. On the other hand, if the culture is first occupied by various widely distributed species of *Aphanomyces* or *Pythium*, whose moist superficial mycelium is very favorable to bacterial development, and whose aerial growth soon collapses to furnish an unimpeded field for feeding, a number of predacious forms may appear in quantity.

The abundance of predacious fungi that appear in isolation plate cultures also depends greatly on the conditions attending incubation. The high temperatures prevailing during summer in Washington, D. C., are generally unfavorable, perhaps mainly because they inhibit the multiplication of infesting animals. Unless, therefore, refrigeration is resorted to neither *Amoeba*-capturing nor nema-capturing fungi are likely to be encountered often during the season in which the isolation especially of organisms causing plant diseases, is most actively carried on. Scarcely less important than temperature is the presence of moisture in available form. A hard culture medium as, for example, maizemeal agar containing over 30 grams of agar-agar to the liter, is often too firm in consistency to permit protozoans or nematodes to force their way through it; besides being sometimes so lacking in free superficial moisture that the animals may be impeded even in their movements on the surface, and perhaps also in a measure starved for want of an adequate supply of bacterial slime. Indeed, even when softer media containing 15 to 20 grams of agar-agar to the liter are employed in Petri dish cultures, *Amoebae* and nematodes and the fungi preying on them flourish much better if surface evaporation is reduced through confinement in a fairly tight container.

In view of the difficulties attending the adventitious development of predacious fungi the frequency with which they yet make their appearance in isolation plate cultures, is remarkable. Almost any bit of decaying vegetable matter that has been in contact with the moist ground for any protracted period of time, can with appropriate handling be made to yield several of them. There is good reason to believe that the fungi herein described as new, by no

means constitute mycological rarities, but deserve rather to be reckoned among the more nearly ubiquitous of plants. Moreover, as the eight species were encountered altogether incidently in the course of only a few months of observation directed primarily toward other objects, it may be presumed that a purposeful search would uncover a much larger number of related forms.

In method of holding their prey the eight species show much uniformity. An *Amoeba* after capture is always to be seen attached, whether to a mycelial element, or, as is often the case in some species, to a fallen conidium, by means of a minute mass of golden yellow adhesive material. From the mycelial element or the conidium is thrust forth a narrow process which passes through the deposit of adhesive material and perforates the animal's pellicle to give rise inside to a more or less characteristically branched haustorium or haustorial system. When the protoplasmic contents of the *Amoeba* are nearing exhaustion, the protoplasm of the haustorium begins to withdraw back into the parent mycelial filament. Eventually the haustorium is completely evacuated and thereupon, like the collapsed pellicle surrounding it, becomes altogether invisible; so that an instance of capture is afterwards found recorded, and then usually only rather dubiously, in a somewhat inconspicuous scar-like or slightly protuberant modification in the contour of the hypha or conidium.

As all attempts to isolate the predacious forms under discussion have failed, it is not known whether adhesive material would be elaborated by them in pure culture, removed from the presence of *Amoebae* as well as from any physical activity simulating the movements of these protozoans. In some of the larger forms the glutinous material may often be clearly seen as minute yellow lumps directly attached to the hyphae at irregular intervals; and even more minute lumps can sometimes be made out though necessarily with greater difficulty, in examining the hyphae and conidia of the more delicate species. On several occasions while observations were being made on the struggles of a captured *Amoeba* to free itself, it was noticed that stretches of the engaged filament in either direction from the prey, on which at first no yellow lumps had been evident, bore unmistakably a number of such lumps an hour or two later. A responsiveness to environmental conditions

is possibly involved here, comparable to that manifested by the predacious Hyphomycetes, all of which have so far consistently failed to produce organs of capture when grown undisturbed in pure culture.

Of living structural parts constituting in their connection with the yellow adhesive material rudimentary organs of capture, the Zygomycetes treated herein offer only a meager and somewhat questionable display. In the several species with sessile bush-like haustorial systems nothing suggestive of prehensile structures have been seen; nor would it seem readily possible that such structures could here be present. However, in species having stalked haustorial systems, short delicate processes with slightly expanded tips have been observed projecting from filaments (FIG. 2, E, F; FIG. 5, J) or from detached conidia (FIG. 5, J; 3, fig. 4, D). These processes correspond closely to the lateral spurs on which newly captured *Amoebae* are held (FIG. 2, A, B, D, E; 3, fig. 4, B, C); and would seem, therefore, to represent special prehensile structures, which after growing through the pellicles of the prey and branching dichotomously inside, come to make up the stalks of the haustorial systems. This interpretation is not necessarily contradicted by the fact that the haustorial stalk is often found wholly inserted in the captured animal (FIG. 2, C, J; 3, figs. 3, B, D; 5, B) since such positional relationship might as readily result from a captured animal engulfing a ready-formed prehensile process, as from the stalk growing into the animal after its capture. Unfortunately the adhesive material that might enable identification of the processes in question as prehensile structures beforehand is generally too minute in quantity to be discerned at all clearly. To add to the difficulties of interpretation, it happens that in species producing conidia directly on prostrate hyphae, the stumps (FIG. 2, B, d; H, b) left after disarticulation of these conidia have approximately the same dimensions as the processes presumed to function in the capture of prey (FIG. 2, E, a-c; F, a-f).

Though the apparatus of capture is in any case extremely simple, it is nevertheless decidedly efficacious in operation,—a circumstance to which the physical feebleness of *Amoebae* generally, combined with the relative firmness and durability of the pellicle recognizable more especially in the terricolous types of these animals, may

contribute in no small measure. Yet the efficacy of adhesive material, unaided by any sort of structural engagement, is revealed not only in *Amoeba*-capturing members of the Zoopagaceae but also in a nematode-capturing member of the family that was figured earlier (2, fig. 8, A, C) and is now more fully discussed elsewhere (7) under the binomial *Stylopage hadra*. Indeed, essentially the same method of capture is known among the predacious Hyphomycetes. For although the stalked, glandular knob-cells in the nema-capturing fungus illustrated synoptically (2, fig. 7, A-C) and subsequently (4) identified as *Dactylella ellipsospora* Grove (8) (= *Monosporidium repens* Zopf (10), = *Monacrosporium leporium* Bubák (1), = *M. elegans* Rostrup (9) non Oudemans), as well as the sessile elongate-ellipsoidal glandular cells produced by the rhizopod-capturing *Pedilospora dactylopaga* Drechsl. (5), are morphologically differentiated organs, they clearly operate altogether by adhesion.

#### SPECIES WITH SESSILE OR NEARLY SESSILE CONIDIA

In five of the species the conidia are borne directly on the hyphae creeping on the surface of the substratum, which make up a large part of the vegetative mycelium. Apparently any superficial hyphal element is capable of giving rise to asexual spores, proximity to the air, and adequate nourishment constituting the only obvious requirements for such reproduction. Each conidium develops as an erect aerial outgrowth from the parent filament. After disarticulation a basal stump remains, which, although longer in some species than in others, is in none worthy of being considered a conidiophore. The lateral attachment of the conidia to the prostrate filaments provides a partial similarity to *Endocoelius asteroides* that is sustained in the presence of terminal appendages on the conidia of some of the forms. Yet in view of the pronounced dissimilarity in morphology of the vegetative thallus, reference to *Endocoelius* seems definitely precluded. A new genus is therefore proposed under a name intended to bring into relief the absence of distinct conidiophores.

#### **Acaulopage gen. nov.**

Mycelium effusum; hyphis continuis, hyalinis, parce ramosis, materia glutinosa flava animalia minuta tenentibus, ramo pelliculam eorum pene-

trantibus, tum haustorium intus evolventibus et carnem vel protoplasma exhaudientibus. Conidia aerea, incolorata, hinc illinc ex hyphis repentibus assurgentia oriunda. Zygosporangia globosa in materia subjacenti e copula-tione duarum similium hypharum orta.

Mycelium spreading; hyphae continuous, hyaline, rather sparingly branched, capturing minute animals by means of yellowish adhesive material, penetrating the pellicle or integument of each by means of a lateral branch, then producing a haustorium within that exhausts the fleshy or protoplasmic contents. Conidia aerial, colorless, arising erect at intervals from prostrate hyphae. Zygo-sporangia globose, produced in the substratum through the union of two similar filaments.

#### ACAULOPAGE RHAPHIDOSPORA

Of the forms eligible for inclusion in the genus perhaps the simplest one morphologically is to be recognized in the species (FIG. 1) having acicular conidia without appendages, that was figured earlier (3, fig. 4, A-E). This species has been seen rather frequently on old agar plate cultures, though it well deserves to be reckoned among the most inconspicuous of organisms. It can be detected most readily by very carefully examining with a dry objective of fairly high magnification the superficial growth present in old plate cultures, especially in areas immediately surrounding the pieces of decaying vegetable material used in starting them originally. The conidia (FIG. 1, E, a-h) in such an examination, are to be discerned as rather sparsely distributed needle-like structures, which, projecting from the surface of the substratum nearly vertically into the air, come into view, for the most part, only endways. For a more satisfactory inspection of the conidia, and for any view of the mycelium whatever, a thin surface layer of the substratum may be sliced off with a moistened razor, carefully removed to a slide, covered with a thin cover-glass, and examined under a water-immersion or oil-immersion objective of high magnification.

In a mount thus prepared the mycelium is seen to consist of filaments (FIG. 1, A-D, F) so delicate that they are exceeded in width by many of the bacteria among which they ramify. The short elements that make up the dichotomously branched haus-torium visible within many of the captured *Amoebae* (FIG. 1,

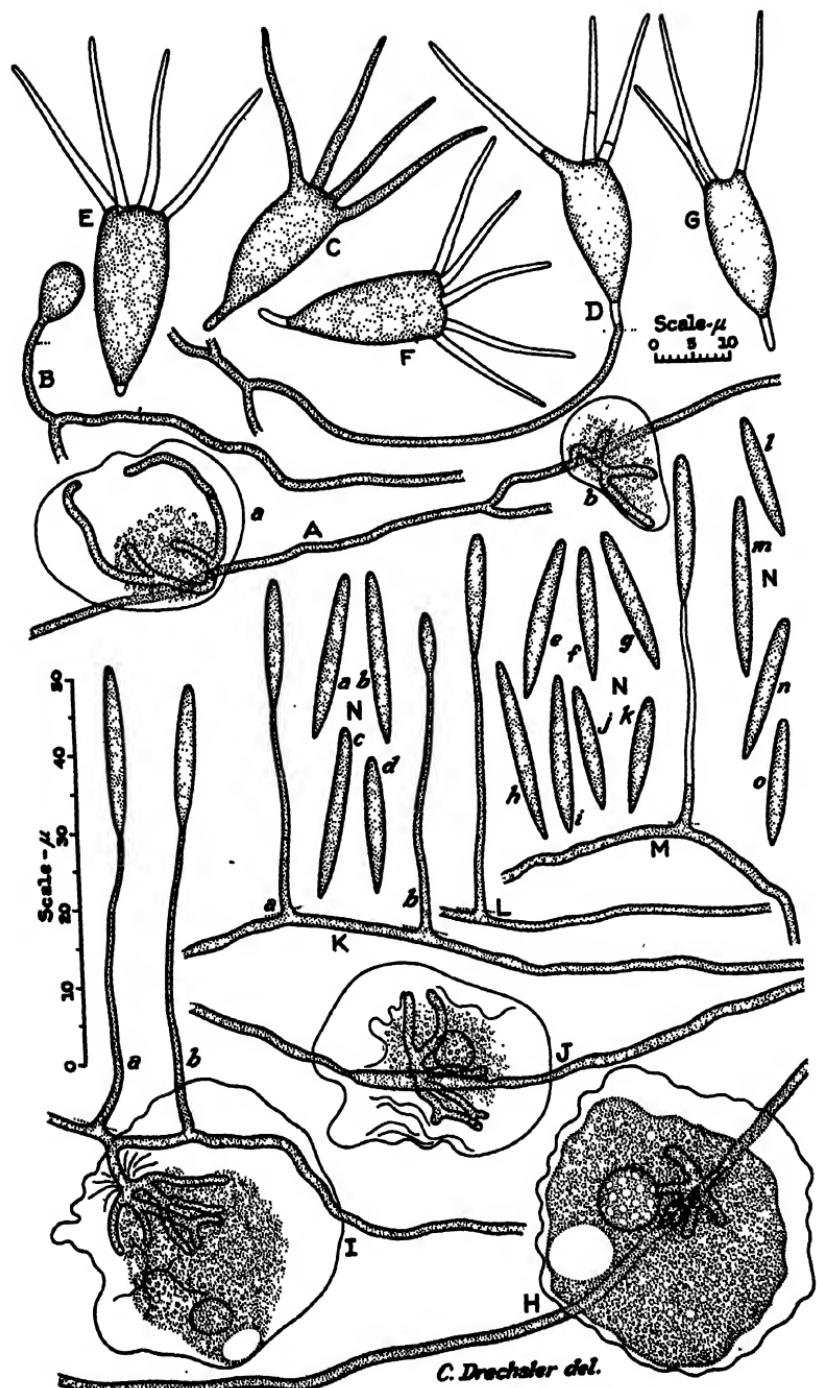


FIG. 4. A-G, *Acaulopage tetraceros*. H-N, *Stylopage haploë*.

A-D) are about twice as wide as the mycelial filaments generally. Haustoria within the larger animals are more extensive and more abundantly branched than those in the smaller ones. The *Amoebae* caught include mostly specimens ranging in diameter between 10  $\mu$  and 25  $\mu$ . In most of the larger newly captured specimens and also in some of smaller sizes, a subspherical nucleus can be made out (FIG. 1, A, C; 3, fig. 4, B). This nucleus together with other less definite features suggests that the usual prey consists of the smaller and the medium-sized individuals of *Amoeba sphæro-nucleolus* Greeff.

Sexual apparatus is formed both on and under the surface of the substratum. Two outwardly undifferentiated hyphal branches meet, fuse at their tips, and from the place of fusion give rise to the globose zygosporangium (FIG. 1, F). A septum makes its appearance in each of the fusing hyphae, though apparently not until the zygosporangium has attained nearly its definite size (FIG. 1, G, H). It appears probable that in spite of this tardiness the delimited portions of filament are approximately homologous to the gametangia of the more familiar Zygomycetes. In any case their contents pass into the zygosporangium in much the same way as the protoplasm of gametangia generally. The zygosporangium thereupon becomes walled off as a spherical cell, and develops internally a zygospore which at maturity has a relatively thick wall with bullate protuberances. Over this sculptured zygospore the slightly relaxed zygosporangial membrane collapses, sometimes rather closely, so that an arrangement of parts very similar to the arrangement in the sexual apparatus of *Zoopage phanera* is brought about (FIG. 1, I-L).

A specific term having reference to the needle-like shape of the conidia seems appropriate for the fungus.

#### ***Acaulopage raphidospora* sp. nov.**

Sparsa; hyphis incoloratis, .6-.9  $\mu$  crassis, haustoria dichotoma ex ramulis 1-1.5  $\mu$  crassis composita evolutentibus. Conidia continua, paulo acicularia, recta vel leniter curvata, 25-45  $\mu$ , saepius 30-40  $\mu$ , longa, 1.2-1.7  $\mu$  crassa. Zygosporangia primo levia, sphæroidea, 5-7  $\mu$  diam., in maturitate membrana circa zygosporam collabente; zygospora incolorata vel flavida, sphæroidea, 4.5-6.5  $\mu$  diam., membrana 5-1.3  $\mu$  crassa, 10-25 verrucis ornata.

Habitat in terra et in materiis plantarum putrescentibus, *Amoebas* minores, saepius 10-25  $\mu$  latas, quae magnam partem probabiliter *Amoebæ sphæro-nucleoli* sunt, capiens et consumens, prope Washington, D. C.

Sparse; hyphae colorless, .6 to .9  $\mu$  wide, producing haustoria consisting of branches 1 to 1.5  $\mu$  wide. Conidia continuous, somewhat needle-shaped, straight or slightly curved, 25 to 45  $\mu$ , mostly 30 to 40  $\mu$  long, and 1.2 to 1.7  $\mu$  wide. Zygosporangium at first smooth, spherical, 5 to 7  $\mu$  in diameter, its wall at maturity collapsing rather closely around the zygospore; the zygospore colorless or yellowish, subspherical, 4.5–6.5  $\mu$  in diameter, with a wall .5 to 1.3  $\mu$  thick and ornamented with 10–25 bullate protuberances.

Occurring in soil and decaying plant materials, capturing and consuming smaller *Amoebae* that measure often 10 to 25  $\mu$  in diameter and belong probably in large part to *Amoeba sphaeronucleolus*; near Washington, D. C.

#### ACAULOPAGE MACROSPORA

A species very similar to the one just described but having generally greater dimensions was found in some quantity in a single agar plate culture. Its mycelial filaments (FIG. 2, A–H), though far from coarse, are approximately twice as wide as those of *Acaulopage raphidospora*. The *Amoebae* that are captured on them and on the conidia include animals larger than any taken by *A. raphidospora*, as well as extremely minute individuals (FIG. 2, A–E, J). They reveal, however, essentially the same morphological features as those preyed upon by *A. raphidospora*, and would seem likewise referable, in the main, to *Amoeba sphaeronucleolus*. Correlated evidently with the generally greater size of the animals captured is a more extensive development of the haustorial system (FIG. 1, A). The haustorial branches are of about the same width as the mycelial hyphae from which they have origin, thus providing a contrast in dimensional relationships with the species already described, wherein the homologous elements are conspicuously wider than the ordinary filaments.

Sexual apparatus was found associated with the fungus, though only in small quantity and in apparently immature condition (FIG. 2, K). As in other members of the group paired branches regularly arise from separate hyphae. The zygosporangium, while half again as large in diameter as that of *Acaulopage raphidospora*, is similarly smoothly spherical on attaining full size, and likewise shrinks somewhat with the contraction of protoplasmic contents incident to the development of the zygospore proper.

Conidia (FIG. 2, I, *a-z*) were produced by the fungus in greater quantity than in any non-catenulate member of the Zoopagaceae observed so far. Examined under a dry objective they stood forth from the substratum in a conspicuously bristling array. In length and in width they exceed the conidia of *Acaulospora rhaphidospora* by approximately a half, and taper less markedly toward apex and base, which as a consequence are more bluntly rounded off. In some conidia the contents were found withdrawn from one or the other of the ends, leaving the empty portion attached as an appendage (FIG. 2, I, *d, f, t, x, z*) comparable, no doubt, with the more distinctive conidial appendages present in other members of the group. Apical bifurcation was seen in some conidia, occurring (FIG. 2, I, *k, r*) apparently as an occasional irregularity.

The sterigmata on which the conidia are borne (FIG. 2, G; H, *a*) represent in this species structures more substantial than in any of the other forms placed in the genus, remaining behind after disarticulation of the spores as tapering projections about  $3\ \mu$  long (FIG. 2, B, *d*; H, *b*). Lateral processes rather similar to them except in being bent or contorted in various ways, were observed on some of the hyphae (FIG. 2, E, *a-c*; F, *a-f*). The difficulty of interpreting these processes has already been referred to. It is not impossible that sterigmata might be constrained into conspicuous irregularity of form through changes in position of the parent hyphae such as might be effected, perchance, by the jostling of young earthworms or of the larger nematodes. Somewhat more plausibility, however, would seem to attach to the explanation that the processes constitute prehensile contrivances, which, after successfully intercepting and holding prey, penetrate into the animals to form the stalked haustorial systems characteristic of the species.

A term having reference to the unusual length of the conidia is deemed appropriate as a specific name for the fungus.

#### ***Acaulopage macrospora* sp. nov.**

*Paulo sparsa*; hyphis incoloratis 1–2  $\mu$  crassis, haustoria divaricata usque ter vel quater repetitive irregulariter bifurcata evolventibus. Conidia elongato-cylindracea, utrimque leniter attenuata et abrupte rotundata, 30–70  $\mu$  longa, 1.6–2.5  $\mu$  crassa, sed interdum sursum bifurcata, etiam interdum parte infera vel parte supra evacuata. Zygosporangia primo levia, sphaeroidea, circiter 9  $\mu$  diam., membrana ad maturitatem circa zygosporam leniter collabente.

Habitat in radicibus putrescentibus, *Amoebas* 5–40  $\mu$  latae, quae magnum partem probabiliter *Amoebae sphaeronucleoli* sunt, capiens et consumens, prope Washington, D. C.

Somewhat sparse; hyphae colorless, 1–2  $\mu$  wide, giving rise to spreading haustoria irregularly dichotomously branched up to 3 or 4 times. Conidia elongate-cylindrical, tapering gradually toward the abruptly rounded basal and distal ends, 30 to 70  $\mu$  long, 1.6 to 2.5  $\mu$  wide, but sometimes distally bifurcate, and sometimes also, with basal or distal portion evacuated. Zygosporangium at first smooth, subspherical, approximately 9  $\mu$  in diameter, with a wall collapsing somewhat about the zygosporangium towards maturity.

Occurring in decaying roots, capturing and consuming *Amoebae* 5 to 40  $\mu$  in diameter, probably belonging mostly to *Amoeba sphaeronucleolus*, near Washington, D. C.

#### ACAULOPAGE RHICNOSPORA

The tendency toward evacuation of a portion of the conidium expressed occasionally in *Acaulopage macrospora*, is manifested with much regularity in a species otherwise closely resembling *A. raphidospora*. When undisturbed material of this species in a Petri dish culture is examined under a dry objective, the conidia directed nearly vertically are seen to terminate individually in a shriveled collapsed prolongation usually somewhat shorter than the basal part (FIG. 3, F, a–c, f–h; 3, fig. 5, C, b), but sometimes equally long or even slightly longer (FIG. 3, F, d). When such material is mounted in water, the empty appendage becomes all but invisible even under the best of immersion objectives, so that its position and size are often revealed only through its interruption of the rather uniformly granular field that the development of bacteria on the surface of the substratum ordinarily provides.

Although the shriveled appendages make for a distinctive appearance, their presence can hardly be considered an altogether decisive diagnostic character. The possibility that the very slender conidia bearing them may represent merely conidia of *Acaulopage raphidospora* that have become evacuated in the distal portions, perhaps through development coming with increasing age, is difficult to dispose of conclusively. However, observations repeated at intervals on stands of acicular conidia devoid of appendages did not disclose apical evacuation on any considerable scale;

whereas in stands displaying appendages apparently in conformity with a usual structural peculiarity, the necessary apical evacuation seemed to have occurred soon after the conidia attained their definitive dimensions. Since, moreover, the appendaged conidia in general slightly exceeded the acicular ones both in total length and in width, it would seem somewhat safer to consider them as being produced by a separate species.

The mycelium like that of *Acaulopage raphidospora* is composed of extraordinarily delicate filaments (FIG. 3, A-D; 3, fig. 5, B). Within the minute *Amoebae* caught on these filaments are produced haustoria, which consist, as in *A. raphidospora*, of a few thicker but relatively short branches borne dichotomously on a short delicate stalk (FIG. 3, A; 3, fig. 5, B). Sexual apparatus is formed readily and in moderate abundance. The fusing filaments arise consistently from separate elements. Sometimes two branches from separate mycelial filaments are represented in the union (3, fig. 5, E, a), sometimes two germ tubes from separate conidia (3, fig. 5, E, b), and sometimes a hyphal branch paired with a germ tube from a conidium (FIG. 3, B-D). The wall of the originally smooth subspherical zygosporangium appears at maturity to collapse closely about the sculptured zygospore proper (FIG. 3, E, a-e); so that in relationship of parts as well as in development, the sexual apparatus offers a rather accurate parallelism with that of *Zoopage phanera*.

A term having reference to the withered aspect of the conidia would seem appropriate as a specific name for the fungus.

#### *Acaulopage rhicnospora* sp. nov.

Sparsa; hyphis incoloratis, .6-.9  $\mu$  crassis, haustoria dichotoma ex ramulis 1.5-1.5  $\mu$  crassis composita evolventibus. Conidia hyalina, 20-55  $\mu$  longa, 1.5-2  $\mu$  crassa, parte supra saepius in maturitate evacuata itaque appendicula marcida constituens, parte infera deorsum attenuata. Zygosporangia primo levia, sphaeroidea, 4.5-7  $\mu$  diam., in maturitate membrana circa zygosporam collabente; zyospora incolorata vel flavida, sphaeroidea, 4-6.5  $\mu$  diam., membrana .5-1.3  $\mu$  crassa, 10-25 verrucis ornata.

Habitat in terra et in materiis plantarum putrescentibus, *Amoebas* minores saepius 10-15  $\mu$  latas capiens et consumens, prope Washington, D. C.

Sparse; hyphae colorless, .6 to .9  $\mu$  wide, producing dichotomously branched haustoria with branches 1 to 1.5  $\mu$  wide. Conidium hyaline, 20 to 55  $\mu$  long, 1.5 to 2  $\mu$  wide, the distal part at

maturity often evacuated and then constituting a withered appendage, the proximal part tapering toward the base. Zygosporangium at first smooth, subspherical, 4.5 to 7  $\mu$  in diameter, its wall at maturity collapsing rather closely about the zygosporae; the zygosporae colorless or yellowish, subspherical, 4 to 6.5  $\mu$  in diameter, with a wall .5 to 1.3  $\mu$  thick and ornamented with 10 to 25 bullate protuberances.

Occurring in soil and in decaying plant materials, capturing and consuming the smaller *Amoebae* that measure mostly 10–15  $\mu$  in diameter, near Washington, D. C.

#### ACAULOPAGE CERATOSPORA

Of all fungi predaceous on *Amoebae* the one that has been observed most frequently is a species of *Acaulopage* having a mycelium only slightly more delicate than that of *A. macrospora*. The *Amoebae* caught on the mycelial filaments (FIG. 3, G–I) include small and medium-sized animals, at least some of which appear to correspond fairly satisfactorily in morphology to *Amoeba sphaeronucleolus*. The haustorium shows a basal, bush-like type of branching rather different from the dichotomous branching characteristic of the three congeneric forms already described. The branches, moreover, are approximately of the same diameter as the mycelial filament from which they arise, providing therefore a contrast to the relationship evident in *A. raphidospora* and *A. rhicnospora* where the haustorial branches are conspicuously wider than the hyphae generally.

Though produced rather sparingly even on well-nourished mycelia, the conidia (FIG. 3, S, a–h) arrest attention both by their dimensions and their distinctive structure. On full maturity, the finely granular protoplasm of the asexual spore is concentrated in an elongated ellipsoidal cell. At its narrow proximal end this cell is delimited by a small septum from a short, narrow, empty basal appendage; at its more broadly truncated distal end it is delimited by a larger septum from a long, empty, tapering appendage. The distal appendage, usually half again or twice as long as the living cell, appears, like that of *Acaulopage rhicnospora*, shriveled when viewed in its natural state on the dry substratum. The evacuated membrane composing it, however, is thick enough in the present

species that it can be made out clearly in a moist preparation under a good immersion objective.

Sexual apparatus is sometimes formed in moderate quantity, but more often is completely absent. It has not been possible to determine definitely whether the fungus is heterothallic; though, as in other members of the genus, the two conjugating branches always arise from separate filaments (FIG. 3, J-L). The fully grown zygosporangium, unlike the homologous structure in *Acaulopage raphidospora* and *A. rhinospora*, is moderately sturdy and rather beautifully ornamented with bullate protuberances (FIG. 3, M-R). As far as can be determined under the optical difficulties introduced by the sculpturing of the zygosporangium, the zygospore proper is surrounded by a smooth spherical wall. Thus, whereas both of the sexual hyphae are connected directly with the zygosporangium, as in *Zoopage phanera*, the sculpturing and eventual shape of mature zygosporangium and zygospore show parallelism rather with *Bdellospora helicoides*.

A term having reference to the hornlike shape of the distal conidial appendage is deemed appropriate as a specific name for the fungus.

***Acaulopage ceratospora* sp. nov.**

Sparsa; hyphis incoloratis, .9-1.8  $\mu$  crassis, haustoria arbusculiformia divaricata ex aliquot ramulis composita evolventibus. Conidia hyalina, in totam 60-110  $\mu$  longa, tripartita: parte supera vacua, sursum attenuata, paulo subulata, 30-70  $\mu$  longa, basi 1-3  $\mu$  crassa, apice .5-.8 crassa, appendicula saepe marcida facta; pars media protoplasmatis viventis repleta, elongato-ellipsoidea, 20-34  $\mu$  longa, 4-6  $\mu$  lata; pars infima vacua, saepe deorsum paulo attenuata, 2-6  $\mu$  longa, .8-1.2  $\mu$  crassa. Zygosporangia flava, sphæroidea, 6-11  $\mu$  diam., 15-25 verrucis ornata; verrucis .5-1.5  $\mu$  altis, 1.5-3  $\mu$  diam. Zygosporae globosae, verisimiliter leves, membrana crassa, loculo interno 4.5-8  $\mu$  diam.

Habitat in terra et in materiis plantarum putrescentibus, *Amoebas* quae pars probabiliter *Amoebea sphaeronucleoli* sunt capiens et consumens, prope Washington, D. C.

Sparse; hyphae colorless, .9 to 1.8  $\mu$  wide, producing bushlike spreading haustoria consisting of several branches. Conidium hyaline, 60 to 110  $\mu$  in total length, consisting of three parts: a distal, tapering, somewhat awl-shaped empty part, present under dry conditions as a withered appendage, 30-70  $\mu$  long, 1 to 3  $\mu$  wide at its base and .5 to .8  $\mu$  wide at its tip; a middle part filled with living protoplasm, elongate ellipsoidal, 20 to 34  $\mu$  long, 4 to 6  $\mu$

wide; a lower empty part often tapering somewhat toward the base, 2 to 6  $\mu$  long, .8 to 1.2  $\mu$  wide. Zygosporangium yellowish, subspherical, 6 to 11  $\mu$  in diameter, ornamented with 15 to 25 warty protuberances, which are .5 to 1.5  $\mu$  high and 1.5 to 3  $\mu$  in basal diameter. Zygospore globose, apparently smooth, and a thick membrane surrounding a loculus 4.5 to 8  $\mu$  in diameter.

Occurring in soil and in decaying plant materials, capturing and consuming *Amoebae* in part belonging probably to *Amoeba sphaeronucleolus*, near Washington, D. C.

#### ACAULOPAGE TETRACEROS

An even more conspicuous development of empty conidial appendages than is found in *Acaulopage ceratospora* provides the chief distinctive feature of a fungus often encountered on old isolation plate cultures, and on pieces of decaying plant materials that have been kept partly bathed in water for some days. In either cultural environment the fungus apparently subsists entirely on *Amoebae*, the animals captured by it being mostly of the smaller sizes. The haustorial system within the prey, is disposed in a bushlike manner somewhat like the haustorial system of *A. ceratospora*, which it resembles besides in that the elements composing it are approximately of the same diameter as the parent mycelial filament (FIG. 4, A, a, b; 3, fig. 2, B).

The conidia, which for the most part are produced rather sparingly, consist individually of a large inversely flask-shaped cell together with a short basal stipe and from 2 to 6, mostly 3 to 5, gradually tapering empty distal appendages. In the earlier stages of its development the conidium first appears as a terminal bulbous enlargement on a very short erect branch arising from a prostrate filament, or on a short erect terminal prolongation of such a filament (FIG. 4, B). From the distal end of the growing enlargement are then thrust forth, in spreading, approximately symmetrical arrangement, the several branches (FIG. 4, C; 3, fig. 2, A) that later through the withdrawal of the protoplasm are converted into the empty subulate appendages. Sometimes apparently this withdrawal is interrupted for a period long enough to entail the laying down of a median partition (FIG. 4, D). On maturity disarticulation occurs a short distance below the point where the

filament widens out. As the short cylindrical part thereby included in the conidium, and comparable to the neck of the inverted flask corresponding to the living cell, has generally been evacuated before disarticulation takes place, it usually presents itself subsequently as the empty basal stipe (FIG. 4, E-G; 3, fig. 2, C) already mentioned.

The conidium thus constituted has an appearance little suggestive of phycomycetous affinities, being reminiscent, even if somewhat vaguely, rather of genera in the Mucedinaceae-Stauropsorae. That at least two fungi eligible for inclusion in the latter group—the quadrilobate *Monacrosporium*-like form figured earlier (2, fig. 9, A, C), and *Pedilospora dactylopaga*—subsist through the capture of microscopic invertebrates, contributes to a remarkable parallelism. It is difficult to avoid the presumption that in some manner the curious modifications in the conidia of these Hyphomycetes, and also the similar modifications in the conidia of the phycomycete under discussion, must be related to the predacious habit that these fungi have in common. In cultures of irrigated vegetable materials, as was pointed out previously, conidia of the present fungus keep afloat on the surface of the water, mainly, no doubt, owing to the buoyancy of the empty appendages. The evident utility of the appendages as floatative devices need, however, not preclude other and perhaps more essential usefulness.

The evident relationship of the fungus especially to *Acaulopage ceratospora* would seem to justify, for the time being at least, assignment to the same genus. A term having reference to four hornlike appendages—four being approximately the average number found, as well as the number most often actually present—is deemed sufficiently accurate in arithmetical connotation to be suitable as specific name.

#### ***Acaulopage tetraceros* sp. nov.**

Sparsa; hyphis incoloratis, 9-1.8  $\mu$  crassis, haustoria arbiculiformia interdum parte dichotoma evolventibus. Conidia hyalina basi stipitata, apice 2-6 (saepe 3-5) appendicibus divergentibus vestita: cellula viventi protoplasmatis repleta, inversum lageniformis, 16-24  $\mu$  (saepius circiter 20  $\mu$ ) longa, 7-10  $\mu$  (saepe circa 8  $\mu$ ) lata; stipite vacuo, 1-5  $\mu$  longo, .8-1.5  $\mu$  lato; appendicibus circum apicem latum cellulae viventis dispositis, vacuis, subulatis, 14-26  $\mu$  (saepe circa 20  $\mu$ ) longis, basi 1-2  $\mu$  crassis. Zygosporae ignotae.

Habitat in terra et in materiis diversis plantarum putrescentibus, *Amoebas* minores quae parte probabiliter *Amoebae sphaerocronucleoli* sunt capiens et consumens, prope Washington, D. C.

Sparse; hyphae colorless, .9 to 1.8  $\mu$  wide, producing bushlike haustoria that sometimes are in part dichotomously branched. Conidium hyaline, stipitate at the base, furnished at the apex with 2 to 6, mostly 3 to 5, divergent appendages: the living cell filled with protoplasm, inversely flask-shaped, 16 to 24  $\mu$  (mostly about 20  $\mu$ ) long, and 7 to 10  $\mu$  (mostly about 8  $\mu$ ) wide; the stipe empty, 1 to 5  $\mu$  long, and .8-1.5  $\mu$  wide; the appendages arranged rather symmetrically about the broad distal end of the living cell, devoid of protoplasmic contents, awl-shaped, 14 to 26  $\mu$  (mostly about 20  $\mu$ ) long, and individually 1 to 2  $\mu$  wide at the base. Zygospores unknown.

Occurring in the soil and in different decaying plant materials, capturing and consuming smaller *Amoebae* that probably belong in part to *Amoeba sphaerocronucleolus*, near Washington, D. C.

#### SPECIES WITH CONIDIA BORNE ON ERECT CONIDIOPHORES

In a number of species closely similar to those described under the genus *Acaulopage* the conidia are borne on erect hyphae, which though not differing much from the vegetative filaments in structural details, are functionally quite distinct in being given up exclusively to asexual reproduction. As has been noted previously these species show nothing of the tendency toward the development of empty conidial appendages evident in *Acaulopage*. It may be inferred with some little plausibility perhaps that in elevating the spore to a position well above the substratum, the ecological need for appendages is obviated. However, even if a divergence in ecological relationship were not to be assumed, the divergence in morphology would yet seem so decisive as to dictate a corresponding taxonomic separation. A separate genus is therefore proposed, under a name intended to bring into relief the presence of conidiophores as well as to make reference to the predacious character that the fungi in question share with members of other genera.

#### **Stylopage gen. nov.**

Mycelium effusum; hyphis sterilibus continuis, hyalinis, parce ramosis, materia glutinosa flava animalia minuta tenentibus, ramo pelliculam eorum

penetrantibus, tum haustorium intus evolventibus et carnem vel protoplasma exhaudentibus; hyphis fertilibus erectis, unicum conidium apice ferentibus vel plura conidia singulatim post incrementa repetita ferentibus. Conidia hyalina, incolorata. Zygosporangia globosa, intra materiam subjacentem e copulatione duarum similium hypharum orta.

Mycelium effuse; vegetative hyphae continuous, hyaline, rather sparingly branching, holding minute animals by means of yellowish adhesive material, penetrating the pellicle or integument of each by means of a lateral branch, then producing a haustorium, or an internal mycelium, which exhausts the fleshy or protoplasmic contents; fertile hypha erect, bearing a single conidium at its apex, or, following repeated elongation, several conidia produced successively. Conidia hyaline, colorless. Zygosporangium globose, produced in the substratum from the union of two similar hyphae.

#### STYLOPAGE IIAPLOE

Of the several known species referable to the genus the one having at once the shortest and simplest conidiophores, and therefore showing the smallest departure in morphology from *Acaulopage*, appears to be relatively scarce, having been encountered only twice on old isolation agar plate cultures. Its vegetative mycelium (FIG. 4, H-M), if slightly more delicate than that of *A. macrospora*, captures *Amoebae* that not only are of approximately the same range of dimensions (FIG. 4, H-J) but also appear assignable in large part to *Amoeba sphaeronucleolus*. The parallelism is extended in the dichotomous branching and limited spread of the haustorial system developed within the prey (FIG. 4, H-J). The conidia (FIG. 4, N, a-o), however, are much smaller than those of *A. macrospora*. They resemble rather closely those of the much more delicate form described herein as *Stylopae araea*, though their somewhat greater dimensions and generally more bluntly rounded apical ends become sufficiently evident as distinguishing features on more careful comparison. Production of successive conidia following repeated elongation of the conidiophore has never been observed in this species; though it might be unsafe to assume that such reproductive development could not occur, for example, in especially well nourished material. A term having reference to the simplicity of the conidial apparatus (FIG. 4, I, a, b; K, a, b; L; M) is deemed reasonably appropriate as a specific name for the fungus.

**Stylopage haploë sp. nov.**

Sparsa; hyphis sterilibus incoloratis, 1-1.7  $\mu$  crassis, haustoria irregulariter dichotoma divaricata evolventibus; hyphis fertilibus incoloratis, 25-40  $\mu$  altis, basi saepius 1-1.2  $\mu$  crassis, sursum paulatim attenuatis, apice .5-.8  $\mu$  crassis, unicum conidium terminale ferentibus. Conidia paulo fusoidea, basi acuta, apice plus minusve rotundata, 15-25  $\mu$  (saepius circiter 19  $\mu$ ) longa, 2.2-2.7  $\mu$  (saepius circiter 2.4  $\mu$ ) crassa. Zygosporae ignotae.

Habitat in materiis plantarum putrescentibus, *Amoebas* saepius usque 40  $\mu$  diam., quae magnam partem probabiliter *Amoebae sphaeronucleoli* sunt, capiens et consumens, prope Washington, D. C.

Sparse; sterile hyphae colorless, 1-1.7  $\mu$  wide, producing irregularly dichotomous spreading haustoria; fertile hypha colorless, 25 to 40  $\mu$  high, 1 to 1.2  $\mu$  wide at the base, tapering upward gradually, .5 to .8  $\mu$  at the tip, bearing a single terminal conidium. Conidium somewhat fusoid, rather acutely pointed at the proximal end, thicker and more or less bluntly rounded at the distal end, 15 to 25  $\mu$  (mostly about 19  $\mu$ ) long, 2.2-2.7  $\mu$  (mostly about 2.4  $\mu$ ) wide. Zygospores not known.

Occurring in decaying plant materials, capturing and consuming *Amoebae* up to 40  $\mu$  in diameter, probably belonging in large part to *Amoeba sphaeronucleolus*, near Washington, D. C.

## STYLOPAGE ARAEA

A fungus showing the same general arrangement of parts as the one just described, but presenting a far different and much more graceful appearance, was observed rather frequently on old isolation plate cultures. Small *Amoebae* as well as medium-sized *Amoebae* measuring up to 50  $\mu$  in diameter and mostly referable apparently to *Amoeba sphaeronucleolus* are captured on its delicate mycelium (FIG. 5, A). The rangy bushlike haustorial system shows close basal branching, supplemented especially in instances of more extensive development in the larger animals with looser branching some distance above the base (FIG. 5, B, C). Haustorial elements and mycelial filaments are approximately equal in width. Undoubtedly the most distinctive feature of the fungus is found in the height and remarkable slenderness of the conidiophore (FIG. 4, D), which at first sight would appear hardly capable of supporting the sizable ovoid conidium (FIG. 5, E, a-z, aa) produced, as far as can be determined, always singly at its tip. A term meaning "slender" is accordingly proposed as appropriate for the species.

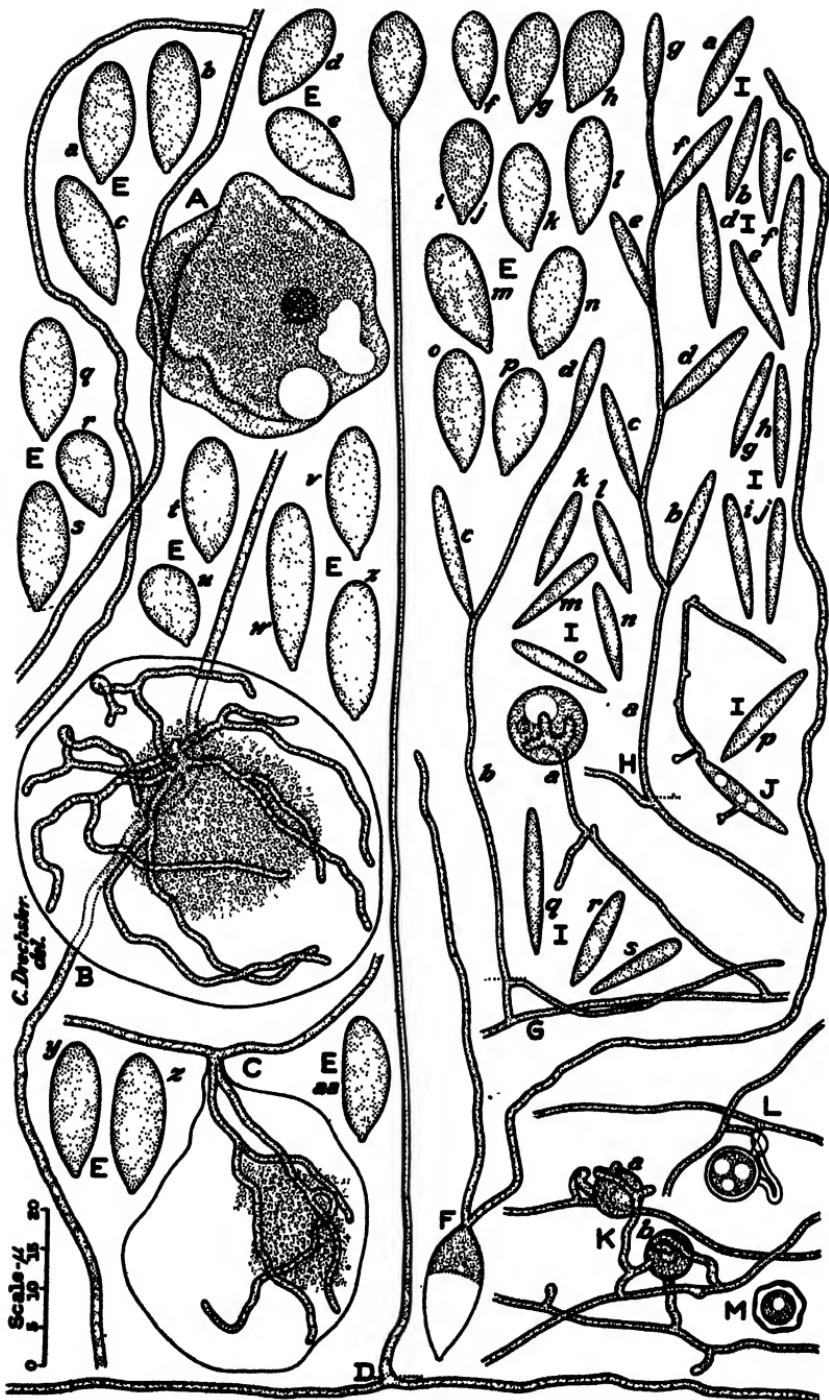


FIG. 5. A-F, *Stylopage araea*. G-M, *Stylopage leptae*.

**Stylopage areae** sp. nov.

Sparsa; hyphis sterilibus incoloratis, .8–1.3  $\mu$  crassis, haustoria divaricata arbusculiformia evolutibus; hyphis fertilibus incoloratis, 150–225  $\mu$  altis, .8–1  $\mu$  crassis, unicum conidium terminale ferentibus. Conidia incolorata, elongato-obovoidea, basi paulo apiculata, 10–22  $\mu$  (saepius circiter 15  $\mu$ ) longa, 5.4–7  $\mu$  (saepius circiter 6.4  $\mu$ ) lata. Zygosporae ignotae.

Habitat in materiis plantarum putrescentibus, *Amoebas* usque 50  $\mu$  diam., quarum multae verisimiliter *Amoebae sphaeronucleoli* sunt, capiens et consumens, prope Washington, D. C.

Sparse; vegetative hyphae colorless, .8 to 1.3  $\mu$  wide, producing spreading bushlike haustoria; fertile hypha colorless, 150 to 225  $\mu$  high, .8–1  $\mu$  wide, bearing a single terminal conidium. Conidium colorless, elongate-ovoid, somewhat apiculate at the base, 10 to 22  $\mu$  (mostly about 15  $\mu$ ) long, 5.4 to 7  $\mu$  (mostly about 6.4  $\mu$ ) wide. Zygospores not known.

Occurring in decaying plant materials, capturing and consuming *Amoebae* measuring up to 50  $\mu$  in diameter, the larger ones apparently belonging to *Amoeba sphaeronucleolus*, near Washington, D. C.

## STYLOPAGE LEPTA

Making its appearance in isolation plate cultures more frequently than either of the two species of *Stylopage* already discussed, is a third form of which figures were included among the synoptic illustrations published earlier (3, fig. 3). As was indicated then the fungus in its vegetative stage closely resembles the two species described herein as *Acaulopage raphidospora* and *A. rhicnospora*; its extraordinarily narrow mycelial threads similarly capturing *Amoebae* of the smallest sizes and producing within each a dichotomously branching haustorium consisting of short widened elements (FIG. 5, G, a; 3, fig. 3, B). Equally close similarity to the two species of *Acaulopage* is evident also in the sexual apparatus, the membrane of the originally smoothly spherical zygosporangium (FIG. 5, K, a, b; 3, fig. 3, E) here likewise collapsing rather closely about the very small sculptured zygospore (FIG. 5, M), and thereby bringing about a relationship of parts much like that described earlier in the account of *Zoopage phanera*. In its asexual reproduction, however, the fungus is moderately distinctive. The erect conidiophores (FIG. 5, G, H; 3, fig. 3, A), in spite of their frail appearance do not stop in their development after producing a single terminal conidium, but through repeated elongation very

often come to bear successively up to a half dozen conidia (FIG. 5, H, b-g) in the arrangement familiar, for example, in *Phytophthora infestans* (Mont.) De Bary. The conidia themselves (FIG. 5, I, a-s) have an obvious resemblance to those of the generally more robust *S. haploë*, but are somewhat smaller and because of their more marked apical tapering have, on the whole, a more distinctly fusoid shape. Besides giving rise to germ-tubes that grow out into delicate mycelia (FIG. 5, J), they often directly produce haustoria (3, fig. 3, D) within *Amoebae* that happen to come in contact with them.

A term having reference more especially to the frailness of the conidiophore would seem appropriate as specific name for this minute inconspicuous fungus.

**Stylopage leptæ sp. nov.**

Sparsa; hyphis sterilibus incoloratis, .6-1  $\mu$  crassis, haustoria dichotoma ex ramulis 1-1.5 crassis composita evolutentibus; hyphis fertilibus incoloratis, 25-100  $\mu$  altis, .7-.9  $\mu$  crassis, usque 6 conidia singulatim post incrementa repetita ferentibus. Conidia fusoidea, basi acuta, ad apicem rotundatum plus minusve attenuata, 12-19  $\mu$  (saepius circiter 15  $\mu$ ) longa, 1.9-2.7 (saepius circiter 2.2  $\mu$ ) crassa. Zygosporangia primo levia sphaeroidea, 5-7  $\mu$  diam. in maturitate membrana circa zygosporam collabenta; zygospora incolorata vel flava, sphaeroidea, 4.5-6.5  $\mu$  diam., membrana .5-1.3  $\mu$  crassa, 10-25 verrucis ornata.

Habitat in terra et in materiis plantarum putrescentibus, *Amoebas* magnam partem 10-20  $\mu$  latas capiens et consumens, prope Washington, D. C.

Sparse; vegetative hyphae colorless, .6 to 1  $\mu$  wide, producing haustoria consisting of branches 1-1.5  $\mu$  wide; fertile hyphae colorless, 25 to 100  $\mu$  high, .7 to .9  $\mu$  wide, bearing up to 6 conidia in succession after repeated elongation. Conidium fusoid, acute at the base, tapering more or less toward the sharply rounded apex, 12 to 19  $\mu$  (mostly about 15  $\mu$ ) long, 1.9 to 2.7 (mostly about 2.2  $\mu$ ) wide. Zygosporangium at first smooth subspherical, 5 to 7  $\mu$  in diameter, its wall at maturity collapsing about the zygospore; zygospore colorless or yellowish, subspherical, 4.5 to 6.5  $\mu$  in diameter, with a wall .5 to 1.3  $\mu$  thick and ornamented with 10 to 25 wartlike protuberances.

Occurring in soil and in decaying plant materials, capturing and consuming *Amoebae* mostly 10 to 20  $\mu$  in diameter, near Washington, D. C.

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## EXPLANATION OF FIGURES

Fig. 1. *Acaulopage raphidospora*; drawn with the aid of the camera lucida at a uniform magnification;  $\times 1000$  throughout. *A-C*, Portions of hyphae with captured *Amoebae*, showing variations in the development of the haustorial system. *D*, Portion of hypha with two captured *Amoebae*, *a* and *b*. *E*, Conidia, *a-h*, showing variations in size and shape. *F*, Zygosporangium, nearly fully grown, at a stage preceding the appearance of septa in the conjugating branches. *G*, Young zygosporangium after appearance of a septum in one of the conjugating branches. *H*, Young zygosporangium after appearance of a septum in both of the conjugating branches. *I, J*, Mature sexual apparatus with hyphal connections, the dotted contour within each representing the optically uncertain outer profile of the zygospore wall. *K, L*, Mature zygosporangia with zygospores, as they appear after their mycelial connections are no longer visible.

Fig. 2. *Acaulopage macrospora*; drawn with the aid of the camera lucida at a uniform magnification;  $\times 1000$  throughout. *A*, Portion of branching hypha on which have been captured four *Amoebae*, *a-d*; showing variations in the dimensions of the animals, and corresponding differences in development of haustoria. *B*, Portion of branching hypha with three captured *Amoebae*, *a-c*, and showing a sterigma, *d*. *C, D*, Portions of hyphae, each with a captured *Amoeba*. *E*, Portion of hypha showing a captured *Amoeba* and three lateral processes, *a-c*, probably representing adhesive organs of capture. *F*, Portion of hypha with six lateral processes, *a-f*. *G*, Portion of prostrate hypha with a growing conidium; the surface of the substratum being indicated approximately in the dotted line. *H*, Hypha with one sterigma, *a*, bearing a fully developed conidium, and another sterigma, *b*, after

removal of conidium. *I*, Conidia, *a-z*, showing variations in size and shape, evacuation of apical (*d, f*) and proximal (*t, x, z*) portions, and distal bifurcation (*k, r*). *J*, Conidium with two minute *Amoebae*, *a* and *b*, captured by it. *K*, Sexual apparatus, showing dichrous origin of two zygosporangia, *a* and *b*.

Fig. 3. Drawn with the aid of the camera lucida at a uniform magnification;  $\times 1000$  throughout.

*A-F*, *Acaulopage rhinospora*: *A*, Portion of hypha with a captured *Amoeba*. *B-D*, Immature sexual apparatus, each zygosporangium being formed from union of a mycelial branch and a germ tube produced by a conidium. *E*, Mature zygosporangia, *a-e*; the dotted contour in each indicating the optically obscure outer profile of zygosporangium wall. *F*, Conidia, *a-h*, showing variations in size, shape, and development of apical appendage.

*G-R*, *Acaulopage ceratospora*: *G, H*, Portions of hypha, each with a captured *Amoeba*. *I*, An *Amoeba* captured and being consumed by two separate hyphae. *J, K*, Sexual apparatus in early stage of development. *L*, Zygosporangium fully grown. *M-P*, Mature sexual apparatus with supporting branches. *Q*, Mature sexual apparatus: *a*, complete, in optical section; *b*, upper aspect of zygosporangial wall alone. *R*, Mature zygosporangium of nearly minimum size. In *M-R* the dotted contour represents the optically uncertain outer profile of the zygosporangium wall.

Fig. 4. Drawn with the aid of the camera lucida at a uniform magnification;  $\times 1000$  throughout.

*A-G*, *Acaulopage tetraceros*: *A*, Portion of hypha with two captured *Amoebae*. *B*, Portion of hypha with conidium in early stage of development, the dotted line indicating approximately the surface of the substratum. *C*, Conidium fully grown but immature, the stipe and appendages still being filled with protoplasm. *D*, Portion of hypha bearing a nearly mature conidium; the septum in each of the two completely evacuated appendages mark an interruption in the process of evacuation at approximately the same stage as that represented in the third appendage. *E-G*, Mature conidia showing variations in size and shape.

*H-N*, *Stylopage haploë*: *H*, Portion of hypha with a rather large captured *Amoeba*. *I*, Portion of hypha with a captured *Amoeba* and two erect conidiophores, *a* and *b*, each bearing a conidium. *J*, Conidium that in germinating gave rise to two germ tubes, in addition to producing a haustorial system within an *Amoeba* captured by it. *K*, Portion of hypha with two conidiophores, one, *a*, bearing a mature conidium, the other, *b*, a young conidium. *L*, Portion of hypha with conidiophore and mature conidium. *M*, Portion of hypha with largely evacuated conidiophore bearing a mature conidium. *N*, Conidia, *a-o*, showing variations in size and shape. The dotted lines in *I, K, L* and *M* indicate the approximate position of the surface of the substratum in relation to the individual conidiophores.

Fig. 5. Drawn with the aid of the camera lucida at a uniform magnification;  $\times 1000$  throughout.

*A-F*, *Stylopage araea*: *A*, Portion of hypha with newly captured *Amoeba*. *B*, Portion of hypha with a well developed haustorial system in the overlying captured *Amoeba*. *C*, Portion of hypha and the haustorial system in a badly depleted *Amoeba*, the attachment being shown in profile. *D*, Conidiophore

arising from a prostrate filament, and bearing a single terminal conidium; the surface of the substratum being indicated approximately by the dotted line. *E*, Conidia, *a-s, aa*, showing variations in size and shape. *F*, Germinating conidium still retaining half of its protoplasmic contents after having given rise to two germ tubes of considerable length.

*G-M, Stylopage leptae*: *G*, Portion of mycelium from which have been produced a haustorium (*a*) within a captured *Amoeba*, and a conidiophore (*b*) bearing a mature (*c*) and a young (*d*) conidium. *H*, Portion of hypha bearing a well developed conidiophore, *a*, with six conidia, *b-g*, formed successively after repeated elongation. *I*, Conidia, *a-s*, showing variations in size and shape. *J*, Germinating conidium, with two delicate lateral processes, probably functional as organs of capture. *K*, Sexual apparatus, showing origin of young zygosporangia, *a* and *b*, from conjugating branches arising from separate hyphae. *L*, Zygosporangium likewise resulting from union of branches arising from separate hyphae, but in somewhat later stage of development. *M*, Mature zygosore with enveloping zygosporangial membrane. In *G* and in *H* a dotted line indicates approximately the position of the surface of the substratum.

# A NEW SPECIES OF CONIDIAL PHYCOMYCETE PREYING ON NEMATODES

CHARLES DRECHSLER

(WITH 1 TEXT FIGURE)

Although the Zoopagaceae hitherto observed in Petri dish cultures started from decaying plant materials consist preponderantly of forms destructive to *Amoebae*, at least several fungi undoubtedly referable to the same taxonomic group have been found that evidently subsist entirely by capturing and consuming nematodes. Of these several fungi the one whose morphology and predacious habit were briefly set forth in the text and synoptic illustrations of an earlier summary (1, p. 140, lines 6 to 13; p. 139, fig. 8, A, C) has made its appearance by far most frequently. In the vicinity of Washington, D. C., it seems to be present on leaf mold wherever in parks or other wooded tracts this material has had opportunity to accumulate in deposits deep enough to retain some moisture during periods of dry weather. When a pinch of leaf mold from such a deposit is added to an agar plate culture already well infested with nematodes, the fungus develops with considerable regularity, giving rise within 5 to 15 days to a growth, which, if ordinarily too scanty to be readily noticed with the naked eye, is fairly conspicuous under a microscope of low magnification.

## MORPHOLOGY, DEVELOPMENT, AND DESCRIPTION

The rather meager mycelium thus revealed is composed of originally continuous hyphae approximately equal in width to the hyphae of the more familiar species of *Aphanomyces*, *Pythium* and *Phytophthora* occurring in diseased vegetable tissues (FIG. 1, A-E). Variations in width are neither frequent nor pronounced, a branch being generally of about the same diameter as the parent filament, and maintaining this diameter without marked diminution well toward its growing tip. Branching occurs at irregular intervals and often at angles approaching a right angle, thus bringing about a characteristically stiff haphazard arrangement of the vege-

tative thallus. The living uninjured hyphae are filled with moderately and uniformly densely granular material, comparable in texture with the protoplasmic contents of the coarser species of *Pythium*, or, perhaps, intermediate in consistency between the protoplasmic material of the genus *Pythium* considered as a whole, and that of the genus *Phytophthora*. The older portions of the mycelium, as in many other filamentous Phycomycetes, undergo progressive evacuation, the retreating contents leaving behind thickish septa at intervals in the empty hyphal envelopes (FIG. 1, C, D). Similar evacuation and deposition of cross-walls takes place also in portions of younger hyphae that have become injured through the protracted and often very violent struggles of captured nematodes (FIG. 1, A).

Capture of prey is effected by means of a yellow adhesive substance similar in appearance to that secreted by the species of *Acaulopage* and *Stylopage* destructive to *Amoebae* (5). Operating in conjunction with this material are definitely differentiated structures in the form of largish globose protuberances (FIG. 1, A, a, b, c). Apparently these protuberances, unlike the stalked adhesive organs of *Dactylaria condida* (Nees) Sacc., are not formed beforehand to await the passage of suitable animals. As they have been found only where nematodes had already been caught, it would seem that their development follows rather than precedes contact with prey. As far as can be determined the animal is first held fast by a local deposit of a sticky substance secreted by the vegetative hypha at a place not otherwise markedly differentiated. In the course of time, as the animal struggles to free itself, there is thrust through the adhesive cake a lateral process, which, upon reaching the integument of the nematode, expands into the globose protuberance. Evidently the thick yellow wall of the protuberance is copiously covered over with adhesive material; so that with the extensive contact afforded by the expanded surface, the animal is held securely. Frequently two or more protuberances participate in catching a nematode (FIG. 1, A, a, b).

Although capture is thus accomplished altogether through adhesion without structural involvement, vigorous eelworms up to 0.5 mm. in length referable to such genera as *Rhabditis*, *Cephalobus*, *Diplogaster*, *Diploscapter*, *Acrobeles*, and *Acrobeloides*, are

held in spite of violent attempts at liberation. In contrast to most nematode-capturing Hyphomycetes, which in a few hours bring about the death of their prey by severing its organs either through intrusion of a bulbous outgrowth, or, more amazingly, through the strangulating action of constricting loops, the present fungus employs no special means of hastening the end of a captured animal. Extensive invasion is therefore of necessity delayed until the captive, after many hours of exertion, has become somewhat quiescent, in the main apparently from exhaustion. Eventually the animal's integument is perforated, and from the adhesive protuberance is intruded an outgrowth that immediately gives rise to hyphae which soon elongate and ramify to permeate the fleshy interior throughout (FIG. 1, A, *d*). The advance of the endozoic hyphae, which are about half as wide as the mycelial filaments, is everywhere reflected in visible degeneration of the organs and musculature of the eelworm. Gradually the degenerated contents become more and more attenuated, until finally they vanish completely. When this process of absorption is nearing completion, the protoplasm in the haustorial filaments begins to migrate back into the mycelial hypha, laying down rather widely spaced septa in its retreat (FIG. 1, B). As the evacuated haustorial system soon becomes largely if not wholly invisible, in the end only the empty and mostly collapsed integument of the nematode is to be seen adhering to the one or more protuberances, which are now walled off from the haustorial elements they had earlier put forth (FIG. 1, A, *e*).

Once a mycelium attains some size it gives rise to a scattering of tall erect conidiophores. These often conclude their development in producing individually a single large ovoid conidium (FIG. 1, C); but with more abundant nourishment they may continue growth from below the first conidium to produce a second farther on (FIG. 1, D, E), and sometimes after repeated elongation to a third, and occasionally even to a fourth. The conidia at maturity (FIG. 1, L-W) drop off on slight disturbance, and then usually without much delay germinate individually by the production of a stout hypha from the apex or from the zone immediately surrounding the basal hilum (FIG. 1, F-K). Despite the readiness with

which the conidia germinate, attempts at growing the fungus in pure culture on maizemeal agar have not been successful.

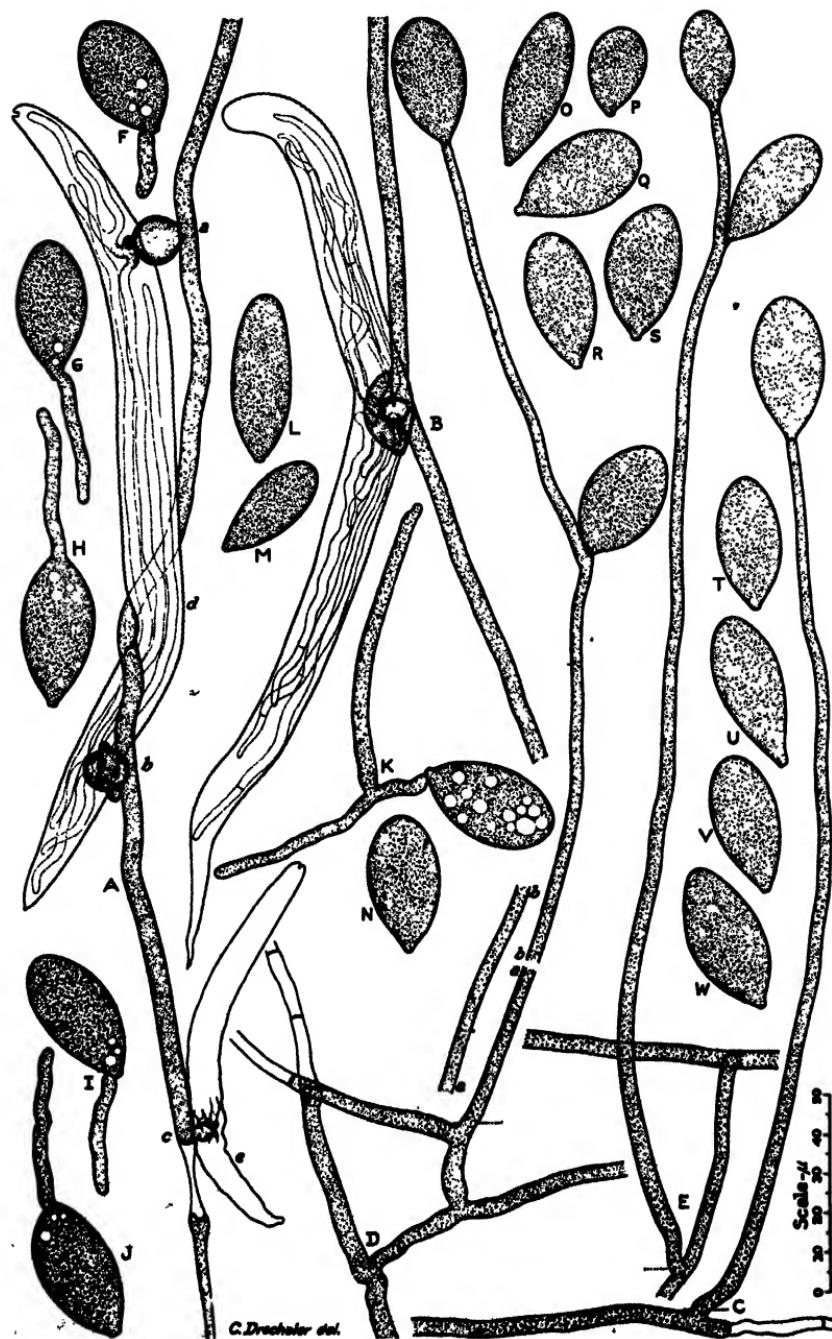
The development of the asexual reproductive apparatus manifestly reveals a close correspondence with the homologous phase in the development of the three known amoeba-capturing species of *Stylopage*. Because of the frequent production of more than one conidium on a single conidiophore the parallelism with *S. leptæ* Drechsl. is especially complete. The great disparity in dimensions might at first seem to compel interpretation of this close parallelism as being fortuitous. However, the differences in size appear far from impossible to reconcile with close relationship of the two fungi, after consideration of *S. araea* Drechsl. In the latter species are expressed, on the one hand, unquestionable similarities in general dimensions and predacious relationships to *S. leptæ*; and, on the other, obvious even if only partial approximation to the nematode-capturing form with respect to stature of conidiophore as well as to size and shape of conidium. The fungus predacious on nematodes is therefore assigned with reasonable assurance to the genus *Stylopage*. A term having reference to its robust stature is deemed appropriate as specific name.

#### ***Stylopage hadra* sp. nov.**

Sparsa; hyphis sterilibus incoloratis, 3.5–5.5  $\mu$  crassis, tubera orbicularia flava glutinosa usque 15  $\mu$  lata et longa evolventibus; his tuberibus animalia tenentibus, integumentum perforantibus, hyphas 2–2.5  $\mu$  crassas intus evolventibus, carnem exhaustientibus. Hyphae fertiles 200–400  $\mu$  altae, basi 4–5.5 crassae, sursum attenuatae, apice 2–2.5  $\mu$  crassae, unicum conidium vel interdum usque 3–4 conidia post incrementa repetita ferentes; conidiis incoloratis, obovoideis, 20–45  $\mu$  longis, 13–23 latis. Zygosporæ ignotæ.

Habitat in terra, in materiis plantarum putrescentibus, praecipue in humo silvarum, nematoda diversa usque .5 mm. longa capiens et consumens, prope Washington, D. C.

Sparse; vegetative hyphae colorless, 3.5 to 5.5  $\mu$  wide, forming yellow adhesive orbicular protuberances up to 15  $\mu$  in diameter, by means of these protuberances holding nematodes, perforating the integument of each, inside producing hyphae 2 to 2.5  $\mu$  wide and assimilating the fleshy contents. Conidiophore 200 to 400  $\mu$  high, 4 to 5.5  $\mu$  wide at the base, tapering upward, 2 to 2.5  $\mu$  wide at the tip, bearing a single conidium, or often producing up to 3 or 4 conidia one by one after repeated elongation. Conidia colorless, ovoid, 20 to 45  $\mu$  long and 13 to 23  $\mu$  wide. Zygospores unknown.

FIG. 1. *Stylopage hadra*.

Occurring in soil, in decaying plant materials but especially abundantly in leaf mold; capturing and consuming nematodes up to .5 mm. long belonging to various species of *Rhabditis*, *Cephalobus*, *Diploscapter*, *Diplogaster*, *Acrobeles* and *Acroboloides*, near Washington, D. C.

#### TAXONOMIC CONSIDERATIONS

The species is apparently not the only representative of its group subsisting on nematodes. Similarities in character of vegetative mycelium and in mode of capture give reason to believe that the predacious fungus with *Pythium*-like intercalary chlamydospores figured earlier (2, fig. 15, D, C) may be closely related to it. A fungus not hitherto referred to, which likewise captures nematodes through adhesion to a continuous mycelium, and which on a short prolongation from the union of two branches coming from separate hyphae gives rise to a zygospore about  $15\ \mu$  in diameter within a closely fitting zygosporangial wall irregularly sculptured with yellow incrustation, undoubtedly represents another member of the group. From these two species, which it is hoped may be more fully discussed after their asexual stages are more completely known, *Stylopage hadra* differs in the moderate and sometimes even rather meager development of its mycelium. This inextensive development finds a plausible ecological explanation in the evident adaptation of the fungus for the capture of the larger and correspondingly more vigorous nematodes. The brisk locomotor movements of these animals insures, on artificial substrata, and presumably also in nature, adequate encounter with prey notwithstanding the moderate extension of the predacious apparatus. Once a relatively powerful animal has been engaged, however, physical sturdiness is required both to hold it securely and to endure the inevitable violence without incurring too severe injury. For although the predacious Hyphomycetes suffer little damage when their organs of capture, together often with connected portions of mycelium, are uprooted, a phycomycete would obviously be more seriously affected if portions of its non-septate thallus were constantly being torn away. Indeed, in spite of the considerable measure of sturdiness attained at the expense of a wider extension, local damage is very frequently plainly evident.

The somewhat inextensive mycelial development, whatever its explanation, brings about an appearance vaguely suggestive of some members of the Entomophthorales. This suggestiveness is sustained in the large size of the conidia, and their similarity in shape to the ovoid conidia described and figured by Thaxter (11) in the presentations more particularly of his *Empusa americana*, *E. montana*, and *E. echinospora*. Occasionally, too, the hypha of germination from a conidium gives rise to a second conidium with so little intervention of a purely vegetative phase that the repetitional development of secondary conidia frequent in many species of the Entomophthorales is approximated. As such repetitional development is fairly widespread among various groups of fungi, occurring for example, in conspicuous measure in many of the predacious hyphomycetous forms referable to *Monacrosporium* and *Dactylaria*, its importance as an indication of affinity hardly merits emphasis. Yet in the absence of all intimate parallelism with any other of the older established groups within the Zygomycetes, the suggestive correspondencies with the insectivorous Entomophthorales, among which a semi-predacious habit of fixing their enfeebled prey to the substratum by means of adhesive substance is frequent, are at least deserving of mention.

Its frequent occurrence in leaf mold and in similar nematode-infested decaying materials, together with the large dimensions of its conidia and conidiophores, would make it seem unlikely that *Stylopage hadra* could have remained unobserved by the numerous mycologists that have devoted themselves to the study of fungi appearing on animal refuse and on decomposing plant remains. Once observed, it might be supposed that the fungus would almost certainly have evoked more than ordinary interest by virtue of morphological features, which, while obviously pertaining to a member of the Phycomycetes, do not conform to those of any of the groups long recognized in that class. That such interest failed to develop may perhaps be attributed less to the fungus having been overlooked than to its probably having been confused with nematode-capturing Hyphomycetes, and of these more particularly with two forms with which it appears in almost habitual association: the fungus with swollen 3-septate conidia and constricting loops figured previously (3, fig. 17, A, C), and possibly

to be identified as *Monacrosporium elegans* Oud. (8); and the fungus with somewhat fusiform 4-septate conidia and stalked adhesive knob-cells (1, fig. 7, A, B, C) corresponding well to Grove's (6) description of his *Dactylella ellipsospora* (4). Very curiously, whether through morphological accident, or, more probably, through what would seem to constitute a remarkable instance of convergence resulting from similarity in predaceous relationship, the two Hyphomycetes mentioned show approximate similarity to *S. hadra* in the dimensions and erect posture of their conidiophores, as well as in the dimensions and shape of their conidia. Their conidiophores, moreover, like the homologous structures of predaceous Hyphomycetes generally, show few septa, and often do not develop these until a relatively late stage. On closer inspection of material in agar cultures, the presence of numerous cross-walls dividing adjacent living cells in the mycelial filaments, and the nearly homogeneous consistency of the protoplasm surrounding the well defined largish vacuoles, are easily recognized as features alien to the fungus under consideration. But when the vegetative mycelium is concealed in an opaque natural substratum, the similarities in habit of the erect aerial parts are brought into deceptively strong relief; so that the conidial apparatus of the Phycomycete might then readily be mistaken for immature apparatus of either of the two Hyphomycetes often accompanying it.

In 1851 Preuss (9) described and figured under the binomial *Menispora ellipsospora* a fungus he found on decaying needles of Scotch fir where it formed thinly effuse growths consisting of white erect non-septate conidiophores bearing individually a single terminal large elliptical spore. According to the description a large oil globule occupied the entire lumen in the median portion of the conidium, which thus came to reveal toward each of its ends a curved contour extending entirely across its width. No mention was made of septa occurring in the conidia of either this species or of *Menispora pyriformis*, which Preuss described at the same time; nor were such septa shown in any of the accompanying figures. The non-septate condition ascribed to the conidia of *Menispora ellipsospora* and *Menispora pyriformis* was emphasized by Oudemans. (8) in distinguishing his *Monacrosporium elegans*

from these species despite the similarity in habit clearly recognized by him. Grove (6) on the other hand considered *Menispora ellipsospora* identical with his *Dactylella ellipsospora*, and therefore cited Preuss' binomial as a synonym. Later Saccardo (10, p. 194) transferred also *Menispora pyriformis* to the genus *Dactylella*. Lindau (7, p. 411-412), though adopting the transfers thus made, commented on the uncertain status of *D. pyriformis*, stating that not even the condition of the conidia, whether continuous or septate, was definitely known.

Since in Preuss' account of *Menispora pyriformis*, at least the conidiophores were described as sometimes containing septa, Lindau's doubts might with even more justification have been directed at *Menispora ellipsospora*. Certainly in its main features the original description of the latter fits *Stylopage hadra* better than *Dactylella ellipsospora* or, for that matter, than any similarly septate hyphomycetous form. However, vacuoles of any considerable size are not usually discerned in the conidia of *S. hadra*, nor in those of the three amoeba-capturing species of *Stylopage*; whereas, very large vacuoles regularly are found in the inflated median cells in the well matured conidia of various predacious Hyphomycetes. In any case the vacuolate condition figured by Preuss, which would perhaps need to be considered rather extreme even for a species of *Trichothecium*, *Monacrosporium*, or *Dactylaria*, appears very definitely foreign to the nematode-capturing phycomycete herein described. This difference in the internal structure of the conidium precludes identification of the fungus with *Menispora ellipsospora* hardly less decisively than the presumptive difference in condition of the conidiophore relative to septation precludes identification with *Menispora pyriformis*. Apart from the two binomials mentioned, the established application of the genus *Menispora* to a distinctive group in the Dematiaceae obviates the possibility of further nomenclatorial or taxonomic involvement with *Stylopage*.

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#### EXPLANATION OF FIGURE

Fig. 1. *Stylopage hadra*; drawn with aid of camera lucida at a uniform magnification;  $\times 500$ . *A*, Portion of hypha on which have been developed three adhesive protuberances, *a-c*; two of which, *a* and *b*, have been operative in the capture and invasion of a rather large nematode, *d*, referable apparently to *Acrobeloides Bütschlii* (De Man, 1885) Thorne, 1925; and the third, *c*, has captured a small nematode evidently of the same species, depleted its contents, and withdrawn the protoplasm from the haustorial elements by means of which the depletion was accomplished. *B*, Portion of hypha with an adhesive protuberance, on which has been captured a nematode belonging to *Cephalobus* sp.; the eelworm is thoroughly permeated with haustorial hyphae, from which, following depletion of the fleshy tissues, the protoplasmic contents are being withdrawn, as is indicated by the appearance of septa near the hyphal ends. *C*, Portion of prostrate hypha bearing a relatively short conidiophore which has given rise to a single conidium. *D*, Portion of mycelium, and arising from it, a conidiophore whereon two conidia have been produced successively; owing to its length the conidiophore is shown in sections, on which *a* and *b* represent corresponding points. *E*, Portion of mycelium from which arises a conidiophore bearing two conidia. *F-K*, Germinating conidia. *L-W*, Conidia showing variations in size and shape.

# A NEW MUCEDINACEOUS FUNGUS CAPTURING AND CONSUMING AMOEBA VERRUCOSA

CHARLES DRECHSLER

(WITH 1 TEXT FIGURE)

In an earlier summary setting forth the morphological features of some fungi that had been found capturing and consuming *Amoebae* in agar plate cultures started from plantings of diseased rootlets and other decaying vegetable materials, was included a brief characterization (3, p. 200, lines 19 to 34; p. 201, fig. 1, A, B) of a septate species predacious on an *Amoeba* then provisionally determined as *Amoeba verrucosa* Ehrenb. The determination can advantageously be retained, since the protozoan, in its relatively large dimensions, its single ellipsoidal nucleus, its slow movement, and its extraordinarily thick pellicle, agrees well with Leidy's description (10) of *A. verrucosa*. Though the animal might also be referred to *A. terricola* Ehrenb. in the broad sense in which that species was understood by Penard (14), it is apparently not identical with any one of the three separate forms to which I have elsewhere (6) applied this binomial together with the numerals I, II, and III, respectively. Of these three forms, it most nearly resembles the one designated as *A. terricola* II, being distinguished therefrom, however, by a different distribution of dark material within the nucleus, and by a much greater thickness of the pellicle (FIG. 1, A). It has been found to develop rather rarely on plate cultures, probably requiring conditions for multiplication not often provided by agar substrata. Owing apparently to this infrequent development, the septate fungus that lives, as far as has been observed, entirely by the capture of the protozoan in question, has put in appearance only a few times.

The mycelium on a transparent substratum like maizemeal agar is similar in general aspect to the mycelium of *Pedilospora dactylopaga* Drechsl., a mucedinaceous fungus known to subsist on shelled rhizopods (5). It reveals a similar sparsely effuse habit

with approximately equally meager branching. The hyphae, which follow rather straightforward courses, in large part on the surface of the substratum, while somewhat wider than the hyphae of *P. dactylopaga*, contain like these, cross-walls separating adjacent living cells, which are filled, except for occasional vacuoles, with protoplasm of fairly homogeneous consistency. At irregular intervals on the hyphae are borne prolate ellipsoidal protuberances that although slightly longer and noticeably more obese obviously correspond to the digitate or elongate-elliptical protuberances of *P. dactylopaga* both in morphology and in function.

An animal on coming in contact with one of the protuberances remains adhering to it, evidently being held by means of glutinous material. Whether the captive makes any effort to escape, apart from movements normally entailed in locomotion, remains uncertain. In any case the substantial pellicle of the animal is perforated when the protuberance puts forth a filamentous outgrowth that penetrates deeply into the protoplasmic interior, at the same time widening gradually in its course. On attaining definitive length, the outgrowth branches dichotomously; the resulting elements very soon bifurcating again (FIG. 1, B), often in planes at right angles to the primary dichotomy (FIG. 1, A). Repeated dichotomous branching follows until the central portion of the animal is occupied by a rather elaborately ramifying apparatus (3, fig. 1, B). This apparatus at first is continuous but later becomes divided by septa into a number of variously shaped segments (FIG. 1, C, a). From these segments, on the depletion of the animal's protoplasmic materials, are put forth narrow hyphae that pass out through the pellicle to extend the predacious mycelium or to give rise to conidiophores and conidia. The pellicle eventually collapses, and persists long as a wrinkled mass testifying to the destructive efficacy of the fungus.

Usually after a few animals have been consumed, conidiophores are produced in small groups scattered here and there on the substratum (FIG. 1, D; 3, fig. 1, A). Relatively short and sparingly branched, they present an atrophied appearance little reminiscent of the stately conidiophores characteristic of most of the nematode-capturing species of *Trichothecium*, *Arthrobotrys*, *Dactylella*, *Monacrosporium* and *Dactylaria*. And the narrow conidia (FIG.

1, D, *a*; E, *a-e*; 3, fig. 1, *A*) borne on these meager reproductive hyphae are correspondingly little suggestive of the conidia produced by the more robust of the hyphomycetous forms preying habitually on eelworms. The empty distal appendage present on the conidium finds no homologue among any of the other Hyphomycetes now known to be predacious, providing instead a striking parallelism with some *Amoeba*-capturing Phycomycetes described elsewhere (7) as members of the genus *Acaulopage*.

Yet the dissimilarities in outward form just noted are hardly such as to preclude a fairly close taxonomic relationship. In the group of *Amoeba*-capturing Phycomycetes, species with well developed conidial appendages are most obviously closely connected with species having only rudimentary appendages, and even with species altogether devoid of such modifications. If the conidiophores of the fungus under consideration are unimpressive in comparison with those of *Pedilospora dactylopaga*, they would seem, judging from Höhnel's original account (9), quite comparable with the conidiophores of *P. parasitans*, a form whose intimate relationship to *P. dactylopaga* cannot readily be questioned. The thoroughgoing resemblance to the latter species with respect to mycelial characters, may therefore be presumed with a fair degree of certainty to indicate membership in the group of closely interrelated predacious Hyphomycetes most familiarly exemplified in *Arthrobotrys oligospora* Fres.

In considering an appropriate disposition of the fungus, this presumptive relationship deserves to be taken into account. If the conidium is regarded as being composed of two cells, it would be difficult to avoid assignment to *Trichothecium*, of which genus three established species, *T. obovatum* (Berk.) Sacc., *T. piriferum* (Fries) Sacc., and *T. inaequale* Mass. & Salm., would seem from their resemblance in habitat, habit, and morphology to the nematode-capturing form figured earlier (2, fig. 10, *A, C*), to represent members of the same predacious series. However, with respect to shape of conidium and to position of the septum within the conidium, these species diverge markedly from the one under discussion. Assignment to *Trichothecium* is further discouraged from the fact that this genus has in large part become familiar to mycologists generally through *T. roseum* Link, a widespread sapro-

phyte and plant parasite that has revealed no predacious tendencies whatever under experimental conditions, and that in morphology is plainly alien to the predacious series.

A more apt disposition in the genus *Dactylella* or *Monacrosporium* is feasible if, as seems permissible, the empty distal conidial appendage is construed as a third cell. Both these genera were erected on species that may rather safely be presumed to belong to the predacious series: *D. minuta* Grove (8) presenting strong similarities in habitat, habit, and morphology to known nematode-capturing forms; while the description of *M. elegans* Oud. (13) except for a somewhat greater length of conidium, applies very well to one of the most abundant and widespread of nematode-capturing forms figured earlier (4, fig. 17, A, C). Like the three established species of *Trichothecium* mentioned, these broad-spored type-species show little family resemblance to the *Amoeba*-capturing fungus; nor is such resemblance greatly evident in the broad-spored nematode-capturing *D. ellipsospora* Grove (= *M. leporinum* Bubák), or in the similarly broad-spored and presumably similarly predacious *D. rhombospora* Grove and *M. ovatum* Petch (15). A closer approximation in general make-up is apparent in the allied forms with narrower conidia, including more particularly *D. minuta* var. *fusiformis* Grove, *M. subtile* Oud. *M. oxytropis* Sacc. & March., and *M. sarcopodiooides* (Harz) Berl. & Vogl., which from their resemblance in habitat, habit and morphology to the somewhat *Fusarium*-like nematode-capturing fungus figured earlier (4, fig. 16, A-C) must be reckoned among the presumptive members of the predacious series.

Saccardo (17, p. 193) early recognized the affinity between *Dactylella* and *Monacrosporium*, but nevertheless held the latter genus distinct from the former because of the presence of copious mycelium. Lindau (11, p. 412) properly regarded the distinction based on the presence of copious mycelium as in itself insignificant, yet adopted it in the belief that whereas the species of *Monacrosporium* probably constitute conidial stages of the coprophilous Sordariaceae, *Dactylella* might more likely be referable to other Pyrenomycetes. An understanding of the predacious habits of the fungi in question places in a different light the substratum relationships on which Lindau's tentative assumption of divergent

pleomorphic connections must have been founded. Moreover, since in pure cultures of such of the predacious forms under discussion as have been isolated, the relative abundance of aerial mycelium is often hardly of sufficient distinctiveness to merit attention in the separation of species, its utility for the separation of genera seems exceedingly doubtful.

The equivalence of the two genera was disturbed more recently when Boedijn (1) extended the application of *Monacrosporium* by describing under the name *M. megasporum* a fungus producing conidia somewhat similar to those of *M. elegans*, but bearing them in closely capitate arrangement. However, as the fungus, evidently an authentic member of the predacious series, answers exactly to the definition of the genus *Dactylaria*, within which it would have found at least one closely related predacious congener, in *D. candida* (Nees) Sacc., and perhaps others in *D. acicularis* Rostrup (16) and *D. pulchra* Linder (12), the extension seems hardly possible of adoption.

In assigning the *Amoeba*-capturing fungus, considerations of priority dictate a preference for *Dactylella*, erected in 1884, over *Monacrosporium*, proposed in 1885 (not apparently in 1884 as is often stated). A term having reference to the knoblike organs of capture is deemed appropriate as specific name.

#### ***Dactylella tylopaga* sp. nov.**

Mycelium sparsum, repens, parce ramosum; hyphis sterilibus 1.5-3  $\mu$  crassis, hyalinis, mediocriter septatis, hinc inde tubera ovoidea vel ellipsoidea 4-7.5  $\mu$  longa, 3.5-5.5  $\mu$  crassa, verisimiliter glutinosa, primo hyalina mox flavida emittentibus, his tuberibus animalia capientibus, pelliculam perforantibus, ramum intus evolventibus; ramo primo hyalino, mox saepe flavente, ad centrum animalium penetrante, sursum paulatim latescente, ramos 2.5-6  $\mu$  crassos repetitive dichotomos mox septatos gerente; his ramis protoplasma consumentibus, hyphas mycelii extus evolventibus. Hyphae fertiles paucae, hyalinae, assurgententes, saepe plus minusve ramosae, 15-50  $\mu$  altae, basi 3-5  $\mu$  crassae, sursum attenuatae, apice 1-1.3  $\mu$  crassae, conidia singulatim gerentes; conidiis hyalinis, in totum 30-50  $\mu$  longis, parte supra eorundem vacua itaque appendicula marcida 13-23  $\mu$  longa, basi 1-1.5  $\mu$  lata, sursum attenuata, apice .5-1  $\mu$  lata facta; parte infera in cellulas duas subaequales, protoplasmatis repletas, 9-17  $\mu$  longas, 2.5-3.5  $\mu$  latas, divisa.

Habitat in humo silvarum *Amoebam verrucosam* capiens et consumens prope Washington, D. C.

Mycelium sparse, creeping, meagerly branched; vegetative hyphae hyaline, 1.5 to 3  $\mu$  wide, moderately septate; at intervals bear-

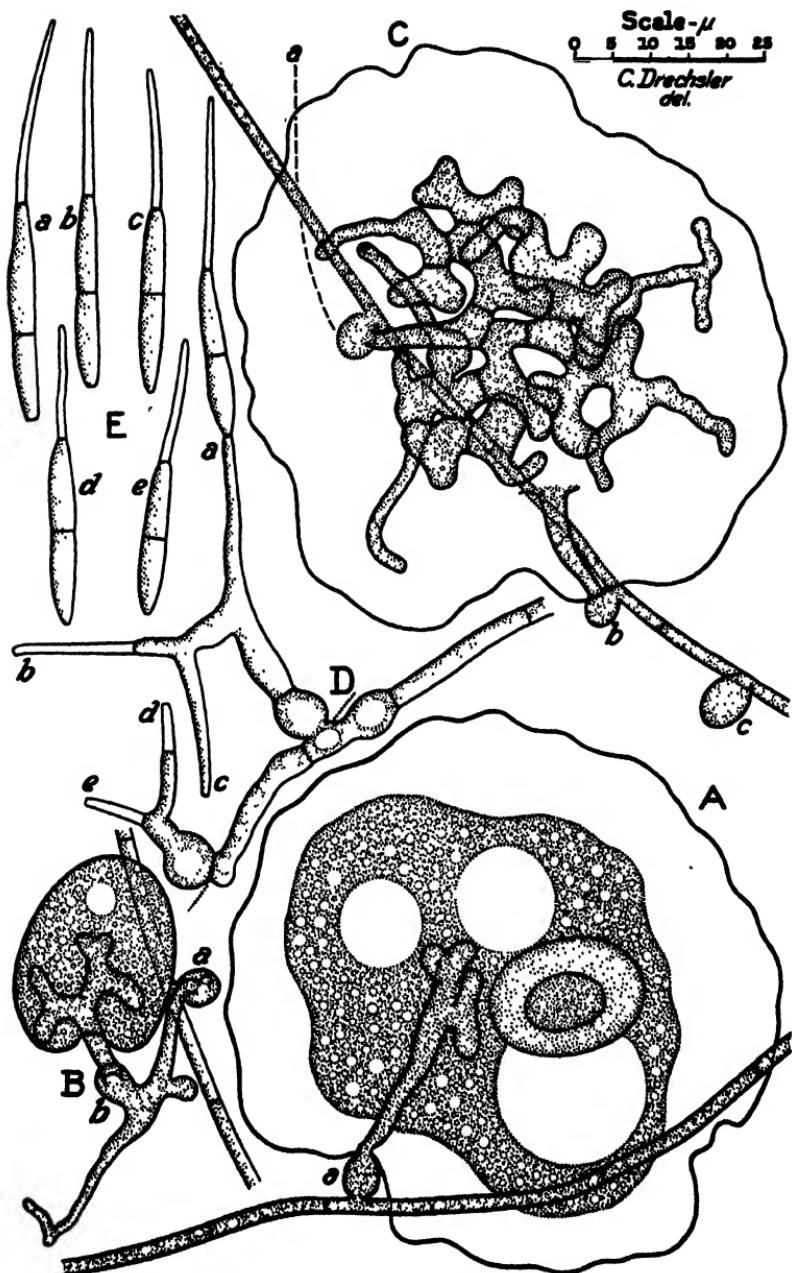


FIG. 1. *Dactylella tylopaga*.

ing ovoid or ellipsoid, apparently adhesive, and ultimately yellowish protuberances, 4 to  $7.5\ \mu$  long and 3.5 to  $5.5\ \mu$  wide; by means of these protuberances capturing animals, perforating the pellicle of each and developing a branch inside; the branch at first hyaline, often turning yellowish after penetrating toward the center of the animal while widening in its course, then giving rise to repeatedly dichotomous branches  $2.5$ – $6\ \mu$  wide, which, though originally continuous, after consuming the animal's protoplasm finally become septate and emit vegetative filaments. Conidiophores few, hyaline, ascending, often more or less branched, 15 to  $50\ \mu$  high, individually 3 to  $5\ \mu$  wide at the base, tapering upward, 1 to  $1.3\ \mu$  wide at the tip. Conidia borne singly, 30 to  $50\ \mu$  in total length, the upper part of each empty and accordingly present as a withered appendage 13 to  $23\ \mu$  long, 1 to  $1.5\ \mu$  wide at the base, tapering upward to an apical width of .5 to 1  $\mu$ ; the lower part divided into two subequal cells filled with protoplasm, each 9 to  $17\ \mu$  long and 2.5 to  $3.5\ \mu$  wide.

Occurring in leaf mold, capturing and consuming *Amoeba verucosa* near Washington, D. C.

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#### EXPLANATION OF FIGURE

Fig. 1. *Dactylella tylopaga* drawn with aid of camera lucida at a uniform magnification;  $\times 1000$ . *A*, Portion of hypha with an adhesive protuberance, *a*, from which a widening branch has been intruded into a captured specimen of *Amoeba verrucosa*. *B*, Portion of hypha with an adhesive protuberance, *a*, that has proliferated an irregular outgrowth bearing a second protuberance, *b*, from which a dichotomously branching process has been thrust into a captured specimen of *A. verrucosa*. *C*, Portion of hypha with three adhesive protuberances, *a-c*, whereof two, *a* and *b*, have captured a specimen of *A. verrucosa*, invaded it extensively, and completely consumed its contents, leaving only the thick empty pellicle; septa have been inserted in the dichotomously branching system, and some of the delimited segments have begun to proliferate ordinary hyphae. (For the sake of clearness the branching development from protuberance *b* is omitted.) *D*, Superficial hypha with two conidiophores, one with three branches, *a-c*, the other with two branches, *a* and *b*; branch *a* being filled with protoplasm and bearing a mature conidium; branches *b*, *d*, and *e* having been partly evacuated. *E*, Mature conidia, *a-c*, showing variations in size and shape.

## NOTES AND BRIEF ARTICLES

### KEY TO SYMBOLS USED BY BERKELEY AND CURTIS IN THEIR COPIES OF SCHWEINITZ' "SYNOPSIS FUNGORUM IN AMERICA BOREALI"

It is the good fortune of American mycologists that there are available in connection with two large collections of fungi in this country the identical copies of Schweinitz' "Synopsis Fungorum in America Boreali" used and annotated by Berkeley and by Curtis when they were studying Schweinitz' specimens. Berkeley's copy is in the Library of the U. S. Department of Agriculture at Washington, and Curtis' copy is in the Farlow Herbarium at Harvard. It is believed that an explanation of the symbols they used in checking the species will be of interest and value to those who may consult these volumes.

Some years ago the writers called attention in this journal (9: 338) to the apparent significance of the symbols used by Berkeley before the species in his copy which are as follows:

H—Specimen found in the Hooker collection at Kew.

C—Specimen loaned him by Curtis.

V—Specimen from Curtis in Berkeley's own herbarium at Kew.

Since this was published, the writers have had opportunity to study Curtis' personal copy of Schweinitz and have made the following key to the symbols used by Curtis:

+ Indicates any Schweinitz specimens he examined.

! + Indicates that part of the specimen was taken by Curtis and loaned to Berkeley. This was to be returned and presumably is to be found in Curtis' Herbarium.

O ! + Indicates part of the specimen was taken and divided with Berkeley and is now in his Herbarium at Kew.

The following equivalence is thus evident:

C Berkeley == ! + of Curtis and means that a specimen should be in the Curtis collection at Harvard.

V Berkeley (in large part = O ! + of Curtis and means that specimens are in both the Berkeley herbarium at Kew and the Curtis' herbarium at Harvard.

Comparison of the two copies checked with numerous specimens in Curtis' herbarium at Harvard and the collection at Kew gives overwhelming evidence as to the correctness of the above interpretation of these keys.

C. L. SHEAR AND N. E. STEVENS

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## THE MYCOLOGICAL SOCIETY OF AMERICA

### REPORT OF THE THIRD ANNUAL MEETING

The third annual mid-winter meeting of the Mycological Society of America was held December 27, 28, and 29 at Pittsburgh, Pennsylvania, in conjunction with that of the American Association for the Advancement of Science. The Society had been granted formal affiliation with the Association during the year, and was represented on the Association Council by our two past presidents, Wm. H. Weston, Jr. and C. L. Shear. The headquarters of the Society were at the William Penn Hotel, one of the largest and finest hotels in the State. This hotel served also as headquarters for the Botanical Society of American, American Phytopathological Society, and other botanical groups. Unusually ample and excellent facilities were afforded for conferences and informal get-togethers among the many botanists thus brought together. The sessions of the botanical societies were held for the most part in the Cathedral of Learning of the University of Pittsburgh, a very tall and exceptionally beautiful building. The arrangements made for the Mycological Society by the local representative, Doctor Otto E. Jennings, were most satisfactory. Favorable weather added to the pleasure of the meeting.

The retiring president, H. S. Jackson, presided at the sessions of the Society, and gave as his address an account of his recent researches on some interesting Heterobasidiomycetes on ferns. The Society held the usual joint sessions with Section G and the American Phytopathological Society. Saturday afternoon was set

aside for the giving of demonstrations of research materials, discussed in the earlier sessions. Interesting displays were made by C. L. Porter of fungi found in apparently fossil condition in Australian sands, by Morris Moore of species parasitic in man and animals, and by S. M. Pady of intracellular mycelium in *Gymnoconia*.

At the regular sessions about thirty mycological papers were presented. Though they dealt with many groups of fungi and many phases of mycology, contributions on cytological and developmental studies were perhaps most outstanding.

At the business session on Thursday morning a committee was named by the president to draft expressions of regret at the passing of several members during 1934. Those removed by death during the year were Charles Fairman, Mrs. Esther Lewis, Thomas H. Macbride, and Frank L. Stevens. New officers elected for 1935 are Bernard O. Dodge, president, John Dearnness, vice-president, and Cornelius L. Shear, councilor. The Council named John A. Stevenson to serve an additional five-year term as associate editor of *MYCOLOGIA*. The editor-in-chief Fred J. Seaver reported on the financial condition of the journal, and announced a plan whereby members may obtain back volumes of *MYCOLOGIA* in exchange for herbarium specimens. Those interested should write to him. The report of the secretary-treasurer shows the finances of the Society to be in good condition. All members are urged to invite graduate students and others to join as associates if regular membership constitutes too heavy an obligation. Associate members do not receive *Mycologia* and may not vote, but they have essentially all the other privileges of regular members including the right to appear on the program. The roll of the Society now includes 338 names, and the membership is slowly growing. The year book for 1935 is now in press and will be mailed early in February.

In response to an invitation from Doctor M. J. Sirks, Honorary Secretary of the Organizing Committee for the Sixth International Botanical Congress, the Council has selected David H. Linder, Fred J. Seaver, and Cornelius L. Shear as delegates to represent the Society in Amsterdam next September.

H. M. FITZPATRICK, *Secretary-Treasurer*

## THE GENUS ZYGOSPERMUM

In a recent publication, "Studies of Coprophilous Sphaeriales in Ontario," Univ. Toronto Studies, Biol. Ser. 38: 73. 1934, the author proposed the name *Zygospermum* for a new genus of Sphaeriales. It has come to my attention that this name has been previously used as a genus by Baillon.<sup>1</sup> Apparently no one except Baillon has taken it up as a genus and he himself reduced it to synonymy a few years later. Nevertheless the name becomes invalid in the sense in which I have used it.

The following new generic name is therefore proposed.

***Zygospermella* gen. nov.**

*Zygospermum* Cain, l.c. p. 73.

***Zygospermella setosa* comb. nov.**

*Zygospermum setosum* Cain, l.c. p. 74.

***Zygospermella insignis* (Mout.) comb. nov.**

*Zygospermum insigne* (Mout.) Cain, l.c. p. 76.

Roy F. CAIN.

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TORONTO, ONTARIO.

## A CORRECTION

Since the publication of the name *Pholiota intermedia* Singer for another species (Beih. Bot. Centr. Abt. 46<sup>2</sup>: 107. 1929), antedates the publication of *Pholiota intermedia* Smith (Ann. Myc. 32: 479. 1934) it is necessary to give a new name to the latter fungus. ***Pholiota septentrionalis* nom. nov.** is proposed.—  
ALEXANDER H. SMITH.

<sup>1</sup> *Zygospermum* Thwaites ex Baillon, Étud. gén. Euphorb. 620, t. 27. 1858.



CHARLES EDWARD FAIRMAN

# MYCOLOGIA

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## CHARLES EDWARD FAIRMAN

1856-1934

HARRY M. FITZPATRICK

(WITH PORTRAIT)

As the Mycological Society of America, in session at Pittsburgh on December 27, was engaged in drafting a formal expression of regret at the loss by death of several of its number during 1934, Charles Edward Fairman, a charter member and one of the oldest individuals in the organization lay dying at his home in Lyndonville, New York. In his passing American mycology loses one of the few students of its Pyrenomycetes possessed of a comprehensive knowledge of species. He was a mycologist of the old school, to whom the study of fungi was merely an avocation. By profession a country physician, he spent practically all the years of his long and busy life in the little hamlet in which he died, remote from mycological centers. His death came suddenly and wholly unexpectedly the day before his seventy-eighth birthday.

Doctor Fairman was born December 28, 1856, in the village of Yates, near the southern shore of Lake Ontario in Orleans County about midway between Rochester and Buffalo. His later home at Lyndonville lies only a few miles to the south. Both of his parents were teachers. From them he must have inherited his love for study and gift for research. During his boyhood his father taught at Yates and Medina, but in 1868

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accepted a call to Shurtleff College at Alton, Illinois, where for five years he was professor of mathematics. In 1873, which was his son's senior year in college, he returned to New York to become principal of Cook Academy at Havana, now Montour Falls. This move resulted in his son leaving Shurtleff before graduation, to enter the University of Rochester. He graduated there in June 1874, when only seventeen years and six months of age. He then entered the St. Louis Medical School, and in 1877 received his M.D. The same year the University of Rochester conferred on him their M.A. Before his twenty-first birthday he was back in the home of his childhood established in the practice of medicine at Lyndonville. He had been an extremely brilliant student, and he was to be outstandingly successful in his profession. As the years passed he became the highly esteemed and much loved family doctor of the whole countryside. In July 1927 the Orleans County Medical Society tendered him a testimonial dinner in celebration of his fiftieth anniversary in the practice of medicine. He gave seven additional years of continuous service before his death.

Doctor F. W. Scott of Medina, a lifelong associate and friend, spoke at the dinner, and in response to my request wrote me as follows concerning the attainments of Doctor Fairman in the field of medicine.

"It is a compliment to be privileged to submit my version of Doctor Fairman's status as a physician. As you suggest, I am aware of his contributions and achievements in research on the fungi, the consideration of which is without my province and with which you are very much more familiar than I am. I also know that this mycological study has been and always will be held in subserviency, and that elevated position and emoluments, financial considerations and freedom from laborious life, have all been renounced that he might remain engaged in his chosen life work, the practice of general medicine. Though he was destined by reason of natural endowments, scholarly attainments, moral excellence and general acumen to become much better than mediocre in any endeavor in which fate might direct him, he never aspired to a specialty in medicine. In fact he deprecated any suggestion or thought of it. For fifty consecutive, continu-

ous years he has and still continues to offer his ministrations to the sick. A significant record of its kind I believe! He is an internist, pathologist, and diagnostician whose worth is recognized beyond the confines of his usual field of labor, and is reflected in the attitude of his loyal, loving clientele and confrères. This then is an estimate of Doctor Fairman; conservative, fair, universal, and not to be confused with posthumous eulogy."

Doctor Fairman was about thirty years of age when he first began to study the fungi. His interest was aroused when he and his father-in-law, Doctor John D. Warren of Lyndonville, undertook to raise mushrooms. He came then into possession of a book on mycology in which he met the sentence: "A wide field is open for research in this branch of botany." The statement spurred his ambition, and led finally to his undertaking serious investigations in the field of the sphaeriaceous fungi. He made contact through correspondence with Ellis, Peck, Saccardo, Rehm, Arthur, and other students in America and abroad, and slowly but surely built up a personal mycological library, and accumulated an extensive herbarium. He obtained most of the general taxonomic works, including Saccardo's *Sylloge Fungorum*; and his herbarium contains various exsiccati which were invaluable to him in his comparative studies. A set of Rehm's *Ascomyceten* was purchased jointly with Elias J. Durand, one taking the Pyrenomycetes, the other the Discomycetes. His contact with Ellis was most fruitful, and numerous species collected by him near Lyndonville, were distributed by Ellis in *North American Fungi* and *Fungi Columbiani*. He described a considerable number of new species and erected a few new genera. His taxonomic contributions were chiefly on the Pyrenomycetes and Fungi Imperfeci. In his later years he specialized somewhat on the Lophiostomataceae, but though his mycological friends urged him to prepare a monographic treatise on this family it was never accomplished. A quotation from one of his letters to the writer is interesting in this connection. He said:

"I do not consider the miscellaneous naming of fungi for other people as a high type of mycological work. When one attempts it he puts himself in the position of describing new things in

groups which need revision, only to find a little later his ground genera disenthroned and the species widely scattered. My reason for writing my paper on 'the fungi of our common nuts and pits' was that I had material on these things which needed to be brought to light. Lloyd complained and so has Doctor Thaxter that I have written so little of a monographic nature, but you know that this type of publication requires access to things a country doctor has not and visits to herbaria and libraries for which he has no time. I wish you to know that I do not look with favor upon any but accurate scientific work, and that opportunity has denied me what I most desire. 'I ne'er shall see fair Carcassonne.'"

Dr. Fairman's modest nature was his outstanding charm. Though he speaks thus disparagingly of his miscellaneous naming of Pyrenomycetes for others, it is well known that few students have been able and willing to attempt identifications throughout this large and puzzling group, though the need for it has been great. In other letters he says:

"I have not been able to complete my work on *Lophiostoma* for lack of time. I thought when I reached my present age no one would wish me for a physician, but it is my lot to have more to do. I put in all my spare time in mycological work. I will not yield to any one in devotion, for I often get up at eleven to one o'clock at night after an hour or two in bed and put in some time on the fungi. I can truly say I have used all available time and strength.

"My publications cannot be in the nature of monographs. I enjoy being a free lance in the field, and while I may run up against Quixotian windmills, sally forth every day to the fray.

"If I have a little time in the morning as I start on my calls I stop in the woods and search for fungi. If I find an interesting specimen I take it with me, and when I reach home for lunch I get out my microscope and study it. If it proves worth while I give it further study that evening, and get it ready for my files."

In November 1918 he wrote:

"I have been engaged in fighting the influenza epidemic. In addition to my private practice I have to attend the duties of the local health office. I have had to see as high as thirty-five

patients a day, and in country practice that means work. I have worked from early morning until 11 or 12 at night and sometimes all night. Therefore, I have not been able to look at the specimens you sent. Though the influenza is now abating I am physically and mentally unequal to the critical study of fungi. I am anxious to again have leisure for my hobby."

Other mycologists, realizing that the life of a country physician is a most strenuous one, have doubtless wondered how Doctor Fairman was able to find time and energy to pursue diligently a scientific study in addition to his time-consuming and exhausting professional duties. These excerpts from his letters reveal that it was only possible because he had a tenacity of purpose and a depth of interest in his mycological endeavors which many others do not possess.

Doctor Fairman was not personally known to a wide circle of American students of the fungi. He travelled little, and only rarely attended botanical meetings. When the occasion offered, it was his custom to make short visits to nearby mycological centers for study amid library and herbarium facilities superior to his own. Perhaps less frequently other mycologists visited him at Lyndonville. Those with whom he thus made contact found him a courteous host and an interesting and charming companion. He was a quiet spoken, unusually unassuming man, of something less than medium height and of somewhat stocky build. The accompanying photograph, taken in a photographer's studio at Medina, shows him at about the age of sixty. It was published by C. G. Lloyd on the cover of *Mycological Notes* No. 60, in 1919, accompanied by a brief statement of appreciation. It is unfortunate that the picture does not show him in his own study surrounded by his books and specimens. The microscope pictured, though old fashioned, was optically excellent, and was supplemented by a thoroughly modern binocular. In other respects he had adequately equipped himself to do accurate work. In some way, probably through his hospital contacts, he had found it possible to imbed his material in paraffin and obtain stained sections when they were necessary.

Some years ago he made a will bequeathing his herbarium and mycological library, including his correspondence of mycological

interest, to his alma mater, the University of Rochester. He was especially desirous that his accumulations should be preserved as an entity. Recent correspondence indicates that his wishes are to be met. As his herbarium contains the type specimens of all of his own species its careful preservation is of course highly desirable.

Doctor Fairman was a member of the Rochester Academy of Science, and a number of his papers were published in its Proceedings. Other contributions appeared in *Annales Mycologici*, the *Journal of Mycology*, and *Mycologia*. In 1920 he published privately at Lyndonville a little pamphlet on the ascomycetous fungi which he had isolated from human excreta. Perhaps his paper of most general interest is that dealing with the fungi occurring on nuts and pits. The following list of his mycological publications is believed to be essentially complete.

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- 1922. New or rare fungi from various localities. *Proc. Rochester Acad. Sci.* 6: 117-139, *pl.* 21-23.

# PARASITISM OF DISPIRA CORNUTA<sup>1</sup>

THEODORE T. AYERS<sup>2</sup>

(WITH 4 TEXT FIGURES)

## INTRODUCTION

The early studies on *Dispira cornuta* van Tiegh., although few in number and limited in scope, showed clearly that the fungus is one of considerable mycologic interest in its structure, reproduction and development and in its relationship and taxonomic position. Of even greater interest is its parasitism, for this fungus has been reported as an obligate parasite on members of the Mucorales, the order to which it, itself, apparently belongs, even though the early investigators using gross cultures only succeeded in demonstrating experimentally that it would parasitize a few unidentified species of *Mucor*. It would seem desirable to determine whether *Dispira cornuta* is parasitic only on species of *Mucor*, or on members of other genera within the Mucorales, on other Phycomycetes, or even on representatives of the other main groups of fungi. Yet any investigation of the parasitism of this fungus necessitates securing it in pure culture, hence, when, as previously reported (2) the writer found that *Dispira cornuta* was not an obligate parasite but could be grown successfully in pure culture on artificial media rich in proteins, a study of this problem was undertaken. In addition certain details of the life history of the fungus both as a saprophyte and as a parasite were examined. The results of these investigations are presented in the following paper.

## HISTORY AND TAXONOMY

*Dispira cornuta* has been collected rarely since it was reported originally from France (1875) by van Tieghem (21), who found

<sup>1</sup> Contribution from the Laboratories of Cryptogamic Botany, Harvard University, No. 108.

<sup>2</sup> At this time, the writer wishes to express his appreciation of helpful criticism, advice, and encouragement to Dr. Wm. H. Weston, Jr., who suggested this investigation.

it growing associated with various species of the Mucorales on rat dung. Since the sporophores of the fungus branched at their tips dichotomously into two equal divisions and the resultant branches bore horn-like growths at the base of the fertile heads, he gave it the appropriate name of *Dispira cornuta*. From North America on rat dung which had been collected in Ohio, a fungus resembling *D. cornuta* was described by Thaxter (18) in 1895. In this American fungus, however, Thaxter observed that the sporophores branched with a false dichotomy; several spore chains were arranged in whorls around the tips of the primary and secondary sterigmata, and furthermore, each spore chain was composed of two spores. In contrast, van Tieghem has described the sporophores of *D. cornuta* as dividing dichotomously at their tips while a single spore chain composed of six spores was attached to the apex of each terminal sterigma. Because of these differences Thaxter concluded that the American fungus was a new species of *Dispira*, and named it *D. americana*. This fungus was considered to be a distinct species until 1906 when Bainier (3) called attention to the errors in van Tieghem's illustrations of *D. cornuta*, and showed that the spore chains in reality comprised two spores each instead of the six spores illustrated, and in reality were arranged in whorls around the tips of the primary and secondary sterigmata. Also Bainier illustrated *D. cornuta* as branching pseudodichotomously. Since these two fungi were so similar in morphological characters, Bainier decided that *D. cornuta* and *D. americana* were synonyms or the same fungus and retained the binomial, *Dispira cornuta* because of its priority. More recently a fungus resembling *D. cornuta* was reported from England (1926) by Elliott (9) as *D. circinata*. According to her, this fungus differed from *D. cornuta* in the following respects: "in having five or six instead of two branches radiating out from the erect conidiophore. The branches bearing the *Aspergillus*-like heads of conidia have the same peculiar method of growth, but in *D. cornuta* the branch which is equivalent in origin to the encircling branch of *D. circinata* is shorter and projects outward in a horn-like fashion. Further in *D. cornuta* each of the globular heads bears only one chain of spores." It should be noted that this description fits

van Tieghem's original and faulty delineation of the fungus rather than Bainier's emendation. However, these variations from the description of *D. cornuta* and the identity of *D. circinata* will be considered in more detail later.

In the fall of 1927, a fungus possessing the essential characteristics of *D. cornuta* was isolated by the writer (2) from growth with an unidentified species of *Mucor* on hog dung which had been collected at Waltham, Mass.. Since this first collection, the same fungus has been collected repeatedly and further study has shown that without doubt it is *Dispira cornuta*. In corroboration it should be noted that Dr. Thaxter examined the fungus and expressed the opinion that it was identical with his *D. americana* and the *D. cornuta* of van Tieghem. The number of collections of this fungus made during the investigation, the dates of collection, the different substrata upon which the fungus and its host grew, are summarized in Table I.

TABLE I  
SOURCE OF COLLECTIONS

Place of Collection	Date of Collection	Substratum	Host
Station No. 1, Waltham, Mass...	Dec. 8, 1927	Hog dung	<i>Mucor</i> sp.
Station No. 2, Cambridge, Mass.	May 7, 1928	Dog "	" "
Station No. 1, Waltham, Mass...	May 27, 1928	Hog "	" "
Station No. 3, Waltham, Mass...	Dec. 8, 1928	Hog "	" "
Station No. 1, Waltham, Mass...	Dec. 7, 1928	Hog "	" "
Station No. 4, Cambridge, Mass.	Dec. 10, 1928	Rat "	" "

Considering the number of collections listed in Table I, it is obvious that *D. cornuta* is more common than hitherto has been supposed. The rarity of this fungus formerly might have been due either to its minute size, its fairly brief appearance in gross culture, or its limitation in nature to those members of the Mucorales which inhabit substrata of high nitrogen content such as dung of rat, hog, or dog. That the fungus was collected repeatedly from the same source without difficulty and at intervals of six months or more seems to indicate that it is fairly persistent in certain localities.

From these collections, isolations of *D. cornuta* and *Mucor* sp. were made, the *Dispira*, even though always considered an obli-

gate parasite, being successfully cultured on nutrient media without a host by methods already described by the writer (2) in a previous paper. After it was learned that *D. cornuta* could be cultivated on certain media in the absence of a mucoraceous host, single spore isolations by the dilution method were made for the purpose of determining if there were different strains among these collections. Even though they were secured from various sources at different times, comparison of the several

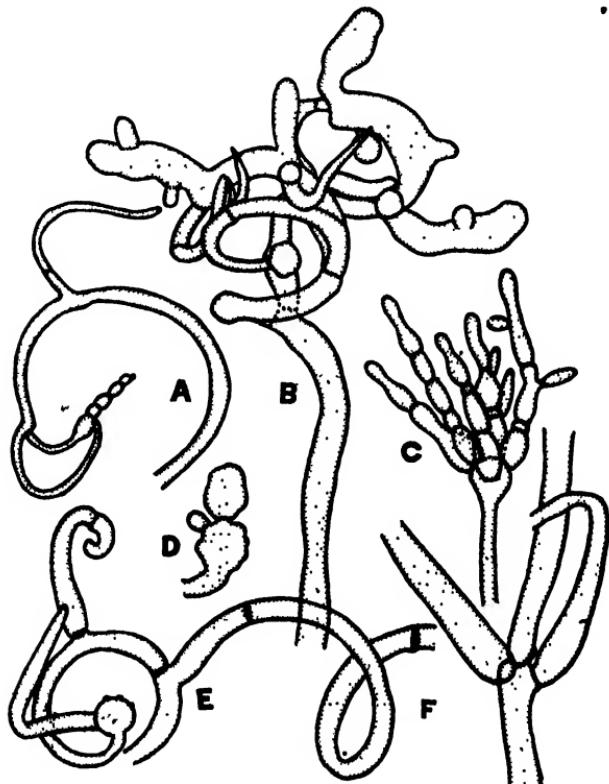


FIG. 1. A, C, D, aberrant forms of fertile heads, sterigmata (A, C 1000 X, D 1700 X); B, young fertile hypha showing proliferation and modification of various parts. 1000 X; E, fertile hypha branching at tip and forming two branches of unequal length. 1000 X; F, fertile hyphae producing three branches instead of the usual number of two. 1000 X.

single spore isolations on the same media under identical conditions failed to show any physiological or morphological differences. It was observed, however, that these isolations were

very plastic in culture, when grown either alone or with a host. For example, under different external conditions such as moisture and humidity, the fertile hyphae (sporangiophores) not only varied in height and produced 2, 3, 4 or more branches (FIG. 1, *f*) of varying length (FIG. 1, *e*), but also branched both dichotomously and pseudodichotomously at their tips. Likewise the fertile heads on these branches (FIG. 1, *c, d, e*) and the horns at the base of these heads (FIG. 1, *b, e*) proliferated considerably and assumed aberrant forms instead of the shapes which are so characteristic of this fungus. Even the sterigmata (FIG. 1, *a, b, d, e*) in some instances became abnormal and resembled van Tieghem's illustrations of a spore chain which showed six spores instead of two for each chain. Nevertheless under no conditions during this investigation were more than two spores in each chain observed. Inasmuch as conditions of culture may induce such decided variations in the branching of the sporophores and the structure of the sporiferous heads it seems clear that the characteristics of these parts, although they have been used for specific identifications are not sufficiently dependable. After becoming familiar with these variations and comparing them with the different descriptions and illustrations given for *D. cornuta*, *D. americana*, and *D. circinata*, it is realized that these three names are synonyms and apply to the same fungus, hence because of its priority, *D. cornuta* should be used.

#### MORPHOLOGY

The several single spore isolations from the various collections of the fungus were not only studied comparatively to see whether they comprised different strains but also were mated on different media either alone or on suitable hosts to determine whether different sexes were present and could be induced to develop a sexual stage. As yet the fungus has never been found to develop any sexual stage whatever. To be sure, Thaxter (18) described the sexual spores or zygospores in 1895 but later he reported (4) that it was the parasitic cells of *Parasitella* on *Mucor* sp. which he had interpreted as zygospores. Despite the fact that hundreds of crosses have been made, using different isolations and subjecting them to various external conditions, no sexual stage has

ever appeared in these cultures. But bodies suggestive of zygosporcs or azygosporcs have been found in various cultures of *D. cornuta* (FIG. 2, c-j incl.). These cells which develop freely even under the usual conditions of cultivation, resemble somewhat the zygosporcs reported for *Blakeslea*, *Choanephora* and *Syncephalis* in their thick walls and large oil globules, but so far the essential stages of conjugation, which occur in the formation of true zygosporcs, have never been observed. At this time, no definite function can be assigned to them with certainty although it seems probable that they enable the fungus to pass through periods unfavorable for growth and sporulation. Besides these bodies just described, chlamydospores (FIG. 2, a, b) are formed by this fungus when it is planted alone on certain media such as malt agar. Their formation probably accounts for the fact that *D. cornuta*, although reduced to a meagre growth of mycelium without sporophores may retain its viability until the agar medium is almost completely desiccated as reported previously.

#### RANGE OF PARASITISM

After securing *Dispira* in pure culture on artificial media it was possible to attack effectively one of the most interesting problems presented by this organism, namely its parasitism.

As pointed out in a previous paper (2) van Tieghem had attempted to culture *D. cornuta* alone on artificial media but had found that although the spores enlarged and produced germ tubes they then remained quiescent. Since, however, he was able to grow the fungus in the presence of various species of *Mucor* he concluded that it was an obligate parasite on members of the Mucoraceae. In the present investigation, therefore, it seemed desirable to determine whether the fungus were parasitic on species of *Mucor* only or could attack other members of the Mucorales and even entirely unrelated fungi of other groups. Accordingly several representative members of the Myxomycetes, Phycomycetes, Ascomycetes, Basidiomycetes, and Fungi Imperfecti were tested as possible hosts for this parasite. To determine whether these various fungi were hosts for *D. cornuta*, pure cultures of these suspected hosts were transferred to tubes of various solid media, usually potato-rat dung-agar, which had

been found to be suitable for the growth of these fungi but not for the growth and sporulation of *D. cornuta*. These cultures were then inoculated with material from pure cultures of *D. cornuta* derived from either single spores or spores and fragments of the fertile hyphae. To make certain that *D. cornuta* had not become adapted to the media used in these tests, a duplicate series of pure cultures of the parasite was transferred to the media used. The cultures of the suspected host inoculated with the parasite and the duplicate series of the parasite alone, were stored in the laboratory under conditions which were suitable for the growth of both fungi, and frequent observations were made on the subsequent growth of these organisms until it was certain that *D. cornuta* was either parasitic or not parasitic on the fungus used in the test.

In Table II are given the fungi tested as hosts, together with the extent of the growth and of the sporulation of *D. cornuta* on those which proved to be susceptible, the extent of growth and the sporulation being described by such terms as trace, meagre, good, etc., and these terms are expressed by symbols in the table as explained in the footnotes. Since this study is not quantitative in nature, these terms suffice to convey to the reader a relative idea of the growth and sporulation of *D. cornuta* on its different hosts. Examination of this table discloses the following points of interest:

*Dispira cornuta* was found to be parasitic on members of the Mucorales only, although as shown in the above table, it was tested on an adequate number of fungi from different groups including representatives from the Myxomycetes (Acrasiales), Phycomycetes (Mucorales, Entomophthoraceae, Saprolegniaceae, and Pythiaceae) Ascomycetes (Endomycetaceae, Phacidiaceae, Dermataceae, and Sphaeriaceae) Basidiomycetes (Ustilaginaceae, Tilletiaceae, Thelephoraceae, Hydnaceae, Polyporaceae, and Agaricaceae), and Fungi Imperfecti (Moniliaceae, Dematiaceae, Phomaceae, and Melanconiaceae).

Within the Mucorales, *D. cornuta* has been found to be parasitic, not only on species of *Mucor*, but on representatives from every known family of this order. These hosts, grouped according to a modification of Lendner's key (14) are as follows:

TABLE II  
HOST RANGE OF *Dispila cornuta*

Fungi <sup>a</sup> Tested as Hosts	Growth of <i>D. cornuta</i>	Sporu- lation	Fungi <sup>a</sup> Tested as Hosts	Growth of <i>D. cornuta</i>	Vege- tative	Sporu- lation
<b>Myxomycetes (Acrasiales)</b>						
<i>Dictyostelium mucoides</i> Bref.	— <sup>b</sup>	—	<i>Endomyces capsulatus</i> Rewbridge, Dodge & Ayers	—	—	—
Phycomycetes			<i>Rhizisma</i> sp.	—	—	—
(Mucorales are not included)			<i>Cucurbitaria</i> sp.	—	—	—
<i>Entomophthora sphacelosperma</i> Fres.	—	—	<i>Scleroderris</i> sp.	—	—	—
<i>Empusa</i> sp.	—	—	<i>Dermatea lindae</i> B. & Br.	—	—	—
<i>Dicyachus</i> sp.	—	—	<i>Biaurella resinae</i> (Fries) Mudd	—	—	—
<i>Pylidium aphanidermatum</i> (Eds.) Fitz.	—	—	<i>Glomerella rufomaculans</i> (Berk.) S. & S.	—	—	—
<i>Physopithora</i> sp. (Mucorales)	—	—	<i>Eutypella</i> sp.	—	—	—
<i>Absidia acerina</i> Banier	XXXX	XXXX	<i>Basidiomycetes</i>			
<i>Absidia glauca</i> Hagem.—plus	XXX	XXX	<i>Sorosporium reticulatum</i> (Kühn) McAlpine			
<i>Absidia glauca</i> Hagem.	XXX	XXX	<i>Entyloma Menispermii</i> Farl. & Trel.			
<i>Rhizopus nigricans</i> Ehren.—plus	XXXX	XXXX	<i>Tilletia laevis</i> Kuehn			
<i>Rhizopus nigricans</i> Ehren.—minus	XXXX	XXXX	<i>Citocybe illudens</i> (Schw.) Sacc.			
<i>Sporadina grandis</i> Link.—minus	XXXX	XXXX	<i>Plenivorus ulmarius</i> (Bull.) Fries			
<i>Circinella umbellata</i> van Tiegh. & Le Monnier	XXXX	XXXX	<i>Hymenochaete</i> sp.			
<i>Mucor</i> sp.	XXXX	XXXX	<i>Irper tulipifera</i> Fries			
<i>Mucor genenerensis</i> Ledin. (Mutant)	XXX	XXXX	<i>Fistulina hepatica</i> Fries			
<i>Zygorhynchus</i> sp.	XXXX	XXXX	<i>Fomes applanatus</i> (P.) Wallr.			
<i>Lichtheimia</i> sp.	XXXX	XXXX	<i>Trametes Pini</i> (Thore) Fries			
<i>Heicostylum elegans</i> Corda	XXX	XXXX	<i>Polyporus cinnabarinus</i> (Jacq.) ex Fr.			
<i>Thamnium elegans</i> Link	XX	XX	<i>Polyporus hispidus</i> (Wulf.) Fries			
			<i>Daedalea quernea</i> (L.) P.			

<i>Chaetostylum Freseñii</i> van Tiegh. & Le Monnier.		Fungi Imperfecti
<i>Pilobolus</i> sp.	xxx	<i>Sepedonium chrysosperma</i> (Bull.) Fries.
<i>Blakesea trispora</i> Thax.—plus.	xx	<i>Oedoccephalum</i> sp.
<i>Blakesea trispora</i> Thax.—minus.	xxxx	<i>Arthrolephium superba</i> Corda.
<i>Lymatia</i> sp.	xxxx	<i>Coemansia reversa</i> van Tiegh.
<i>Chaetododsum Brefieldii</i> van Tiegh. & Le Monn.	xxxx	<i>Verticillium asparicinum</i> (Link) Corda.
<i>Mortierella I</i> (dead wood).	xx	<i>Penicillium glaucum</i> Link.
<i>Mortierella II</i> (Brazil nut).	xxxx	<i>Gliocladium</i> sp.
<i>Mortierella III</i> (Seattle, Wash.)	—	<i>Aspergillus candidus</i> (P.) Link.
<i>Mortierella IV</i> (yellow).	x	<i>Clonostachys Cneorum</i> Oudem.
<i>Mortierella V</i> .	—	<i>Alternaria</i> sp.
<i>Mortierella echinulata</i> Harz.	—	<i>Macrosporium</i> sp.
<i>Channephora infundibulifera</i> (Currey) Sacc.	? *	<i>Bostrychus</i> sp.
<i>Channephora cucurbitarum</i> (Berk. & Rav.) Thaxter—plus.	xx	<i>Cephaliophora tropica</i> Thax.
<i>Channephora cucurbitarum</i> (Berk. & Rav.) Thaxter—minus.	x	<i>Trichurus</i> sp.
<i>Cunninghamella echinulata</i> Thax.—plus.	?	<i>Dematioides pululans</i> Barry.
<i>Cunninghamella echinulata</i> Thax.—minus.	xx	<i>Cladosporium herbarium</i> (Pers.) Link.
<i>Pipacephalais</i> sp.	?	<i>Isoetes</i> sp.
<i>Syncephalastrum I</i> (Porto Rico).	—	<i>Coremium</i> sp.
<i>Syncephalastrum II</i> (Panama-sloth dung).	xxxx	<i>Fusarium</i> sp.
	xxxx	<i>Sphaeropssis Ellisi</i> Sacc.
	xxxx	<i>Pestalotia funerea</i> Desm.

Acknowledgment is made at this time to those who kindly gave the writer cultures of various fungi for use in this investigation.

**Explanation of symbols:** No growth = —; Trace = x; Meage = .; Fair = xxx; Good = xxxx.

\* In the case of these particular hosts, it was difficult to distinguish the vegetative hyphae of the parasite from those of the host because of their macroscopic similarity.

**A. Sporangial forms:**

Mucoraceae—*Absidia glauca*, *A. caerulea*, *Rhizopus nigricans*, *Sporodina grandis*, *Circinella umbellata*, *Mucor* sp., *Mucor* sp. (Norway), *Mucor genevensis* (Mutant),<sup>3</sup> *Lichtheimia* sp.

Thamnidziaceae—*Helicostylum elegans*, *Thamnidium elegans*, *Chaetostylum Fresenii*

Pilobolaceae—*Pilobolus* sp.

Mortierellaceae—*Mortierella I*, *Mortierella III*

**B. Conidial forms:**

Chaetocladiaceae—*Chaetocladium Brefeldii*

Choanephoreaceae—*Choanephora infundibuliforme*, *C. cucurbitarum*, *Blakeslea trispora*, *Cunninghamella echinulata*, *Lymania* sp.

Cephalidaceae—*Syncephalastrum I* (Porto Rico), *Syncephalastrum II* (Panama).

Although *D. cornuta* was not equally parasitic on all species of the Mucorales, it was observed to be equally parasitic on the plus and minus strains<sup>3</sup> of *Rhizopus nigricans*, *Absidia*

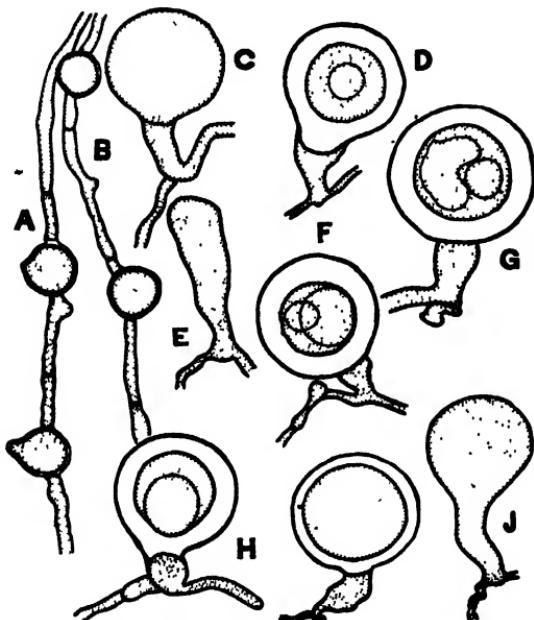


FIG. 2. A, B, chlamydospores formed by *D. cornuta* on "Difco" bacto-wort agar. 1000 X; C, G, zygosporule-like bodies formed by *D. cornuta* growing on *Mucor* sp. 1700 X; D, F, H, mature zygosporule-like bodies formed by *D. cornuta* growing alone on egg medium. 1700 X; E, I, various stages in the formation of zygosporule-like bodies when *D. cornuta* grew as a parasite on *Mucor* sp. on potato-deer dung agar. 1700 X; J, immature zygosporule-like body formed by *D. cornuta* growing alone on egg medium. 1700 X.

<sup>3</sup> Obtained from Dr. A. F. Blakeslee.

*glaуca*, *Cunninghamella echinulata*, and *Blakeslea trispora*, but more parasitic on the minus than on the plus strain of *Choanephora cucurbitarum*.

It is interesting to note that *D. cornuta* although parasitic on members of the Mucorales is very limited in its parasitism on members of the genus *Mortierella*. For example, it was found to be actively parasitic on *Mortierella* I, weakly parasitic on *Mortierella* III, but not parasitic at all on *Mortierella* II, *Mortierella* IV, *Mortierella* V, and *M. echinulata* or on various collections of the latter fungus from different sources.

In addition, *D. cornuta* was found incapable of using *Piptocephalis* sp. as a host. Similarly *Dispira* was found not to be parasitic on *Coemansia reversa*, which by many has been considered as a member of the Mucorales. The possible bearing of these results on the taxonomic position of *C. reversa* will be discussed later.

#### METHOD OF INFECTION OF HOSTS

Because of the simple structure of the host and the equally simple form of the parasite, an opportunity presented itself to study such points as the method of penetration, the origin of the infecting organs, the type of the parasitic organs, and the part of the host attacked. To study these points effectively, *Sporodinia grandis* was used as a host because its mycelium could readily be distinguished from that of *D. cornuta* by the greater diameter of its hyphae, its brown color, and by its lack of the disc-like septa which occur in *Dispira*. In this study, hyphae and spores from pure cultures of both host and parasite were added to hanging drops of proteose-peptone solution (1.5%). These cultures were stored in the laboratory under conditions suitable for growth of both host and parasite. Subsequent examination of these cultures showed that the spores of *D. cornuta* germinated within 24 hours, and in some cases germinated even before they were completely separated from each other in the spore chains. The first indication of germination was the increase in the size of the spores which swelled laterally until they became globular or almost so in shape (FIG. 3, b, c, d). After increasing in volume several times, they then produced one or

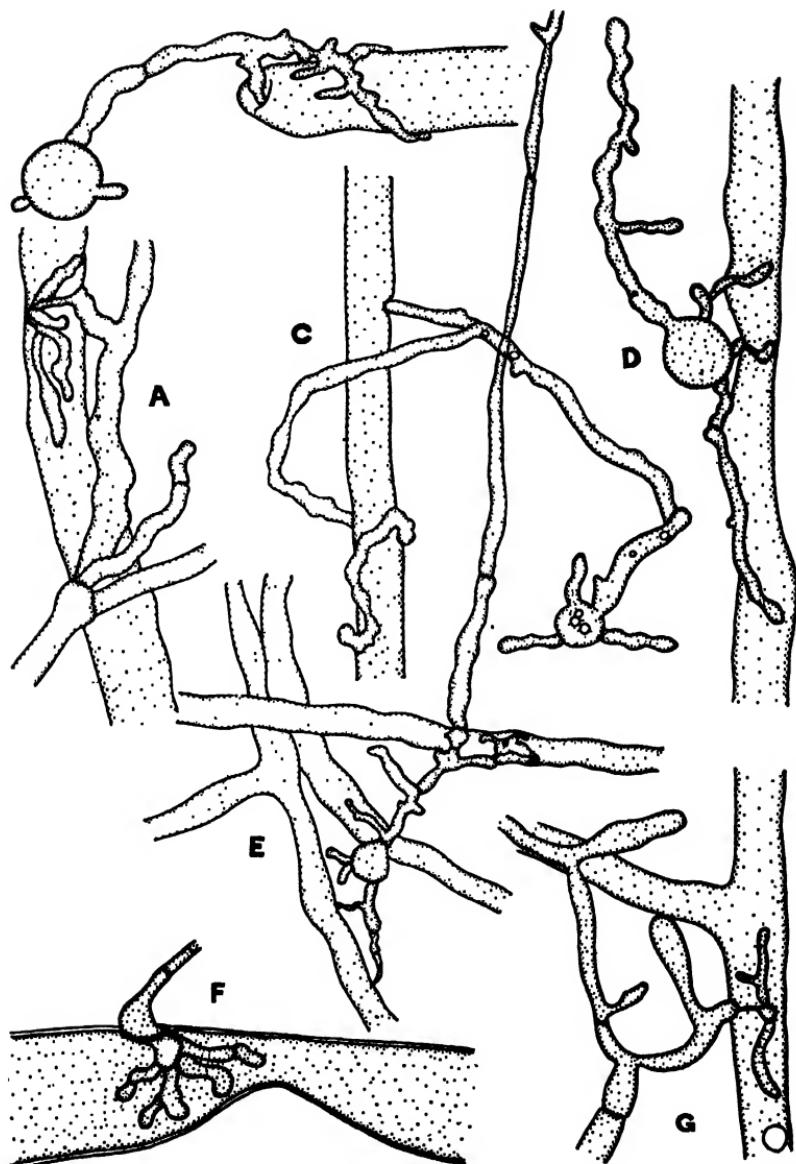


FIG. 3. A, G, haustoria formed by *D. cornuta* within the hyphae of *Sporodinia grandis*. 1700 X; B, C, D, germ tubes from spores of *D. cornuta* beginning to penetrate hyphae of *S. grandis*. Note the germ tube pressing in the wall of the hypha of the host in the case of B and D. B, D 1700 X, C 1000 X; E, branches of germ tube of *D. cornuta* penetrating a hypha of *S. grandis*. In one instance, a haustorium has already been formed. 1000 X; F, haustorium of *D. cornuta* in an old hypha of *S. grandis*. 1200 X.

more usually terminal germ tubes, or in some cases lateral as well (FIG. 3, b, c, d, e). These germ tubes lengthened, became septate and finally branched. Eventually some of the tubes or their branches reached the vegetative hyphae of the host, usually forming at the point of contact, knob-like appressoria (FIG. 3, a, d, f, g), from which a slender constricted tube entered the interior of the host. In a similar manner the fungus spread, developing its extensive mycelium, attaching itself to the hyphae of the hosts, and forming more haustoria within the host cells.

Once penetration within the wall of the host has been accomplished, the penetrating tubes branch to form finger-like haustoria. Seemingly the fungus gains entrance into the host by a combination of chemical and mechanical action, since in some instances the walls of the host have been observed to be indented (FIG. 3, b, c, f), at the points of entrance, while in others the wall is not indented (FIG. 3, a, d, e, g) and the hyphae of the parasite are constricted considerably when passing through the host walls to the interior. These observations on the host and parasite growing in hanging drops were corroborated also in a study of the two organisms growing together on various solid media. So far, haustoria have been observed only in the vegetative hyphae and never in the sporangiophores, zygospores, or aerial hyphae of the different hosts. Also in the hanging-drop cultures, it was found that the parasite was unable to penetrate the walls of the host, *Sporodinia grandis*, when they became thickened and brownish in color, and fully mature.

The haustoria formed by *D. cornuta* in the cells of *Sporodinia grandis* resembled somewhat those of this same parasite in *Mucor* sp. as illustrated by van Tieghem, but the mycelium of the parasite was observed not to be so sharply differentiated into the creeping position and the upright fertile hyphae shown by him. The parasitic organs resembled those of various fungous parasites of the higher plants, for example, the Erysiphaceae, Ustilaginaceae, and Uredinales rather than the complex "cup-cells" (Schröpfzelle) reported for the Mucoraceous parasites *Chaetocladium* and *Parasitella* by Burgeff (8) or the haustoria of the various species of *Piptocephalis* or *Synccephalis* on different hosts as illustrated by van Tieghem (21), Brefeld (5), and others.

THE INFLUENCE OF MEDIA ON THE PARASITISM OF  
DISPIRA CORNUTA

In studying the parasitism of *D. cornuta* on certain fungi, it was observed that *D. cornuta* either failed to parasitize these hosts or grew very meagerly on them when they were grown on agar media containing chiefly sugars as prune, oatmeal, cornmeal, and malt media. In contrast, when these same hosts were cultured on media such as nutrient bean pod and peptone agars consisting mostly of proteins or their derivatives, *D. cornuta* either completely covered the colonies of the host with its growth, or formed on them a large, conspicuous tuft of vegetative hyphae and sporophores. These results suggested that the presence of certain carbohydrates or proteins in the medium had a marked influence on the susceptibility or resistance of these hosts to *D. cornuta*. Therefore, tests were made to determine whether proteins and sugars actually affected the susceptibility or resistance of certain hosts to this mucoraceous parasite. For hosts in these experiments, *Mucor* sp. and *Mortierella* I were used because they had been found to be susceptible to *D. cornuta* and because their scanty growth did not mask that of the parasite. These hosts were cultivated on two types of agar media one of which contained only proteose-peptone and the other only dextrose as nutrients, varying quantities of these substances being used to determine if the concentration would affect susceptibility and resistance. In these tests *Mucor* sp. and *Mortierella* I were first transferred to the media and then approximately equal masses of inoculum consisting of the mycelium, sporophores and spores of *D. cornuta* were added. The results of these tests, together with the composition of the different media used, are embodied in Table III.

It is obvious from Table III that the kind of nutrient rather than the quantity of nutrient in each medium affected the susceptibility of *Mortierella* I and *Mucor* sp. to *D. cornuta*. For example with *Mortierella* as the host, *D. cornuta* completely covered the compact, irregular, colorless, bacterioid colony of *Mortierella* I (FIG. 4, d), with an almost solid mass of sporophores when this host was cultivated on proteose-peptone medium of different concentrations. In contrast, when this same host was

cultured on media containing only different quantities of dextrose as a nutrient, *D. cornuta* did not develop at all although the *Mortierella* formed limited mycelioid colonies about 35 mm. in diameter composed of delicate colorless hyphae only faintly visible to the unaided eye (FIG. 4, c).

TABLE III  
INFLUENCE OF MEDIA ON THE PARASITISM OF DISPIRA CORNUTA

Medium used	Growth of <i>D. cornuta</i> on <i>Mortierella</i> I	Growth of <i>D. cornuta</i> on <i>Mucor</i> sp.
Proteose— Peptone Agar <sup>1</sup> (4, 8, 12 or 16 gms. of nutrient per L.)	<i>D. cornuta</i> covered com- pletely the bacterioid col- ony of <i>Mortierella</i> I with a dense growth of mycelium and sporophores (Fig. 4, d).	<i>D. cornuta</i> formed a patch of sporophores about 3-4 mm. in diam. on the dense, brown colony of <i>Mucor</i> sp. (Fig. 4, a).
Dextrose Agar <sup>1</sup> (4, 8, 12 or 16 gms. of nutrient per L.)	No growth of <i>D. cornuta</i>  <i>Mortierella</i> I formed a colony about 35 mm. in diam., composed of deli- cate, hyaline hyphae, scarcely visible to the naked eye (Fig. 4, c).	No growth of <i>D. cornuta</i>  <i>Mucor</i> sp. covered entire surface of medium with a grayish, wooly mycelium (Fig. 4, b).

<sup>1</sup> 2 percent agar used.

Similar results were secured when *Mucor* sp. and *D. cornuta* were cultivated on dextrose and proteose-peptone media. Here again the nutrient rather than the quantity used in the media affected the susceptibility of *Mucor* sp. to *D. cornuta*. When this host was grown on media containing proteose-peptone in different quantities, *D. cornuta* made on it rather limited growth in patches about 4 X 3 mm. in diameter (FIG. 4, a) while the *Mucor* sp. itself covered the entire surface of the medium (40 mm. in diam.) in all cases with a compact mycelial mat which was gray at first but became light brown with sporangial production. In contrast, *D. cornuta* failed to develop at all when *Mucor* sp. was growing on dextrose media while the host spread over the entire surface of this type of substratum, forming a colony about 40 mm. in diameter, with a pale, smoke-gray color (FIG. 4, b), again demonstrating that the kind of nutrient rather than the quantity affected the susceptibility of a host to *D. cornuta*.

To determine if the *D. cornuta* affected the growth of either *Mucor* sp. or *Mortierella* sp. on these media, a separate series of cultures of these hosts were started from the same source simul-



FIG. 4. Upper left, *D. cornuta* growing parasitically on *Mucor* sp., proteose-peptone agar used; upper right, *Mucor* sp. growing on dextrose agar, note the absence of *D. cornuta*; lower left, *Mortierella* I on dextrose agar, note the absence of *D. cornuta*; lower right, *Mortierella* I on proteose-peptone agar. Note that *D. cornuta* has completely covered the colony of this host.

taneously with the inoculated cultures. In these it was observed *Mucor* sp. and *Mortierella* sp. produced colonies of the same type and approximately the same size as in the cultures inoculated with *Dispira*, thus demonstrating that although *D. cornuta* is parasitic on these hosts, it does not impede their growth.

After the foregoing experiments had been repeated several times and the same results had been secured, it seemed conclusive that the composition of the medium affected the susceptibility and resistance of *Mortierella* sp. and *Mucor* sp. to *Dispira cornuta*.

However, further tests were made to determine if these conclusions were true for other organisms. In these later experiments *Mucor genevensis* (Mutant) and *Mucor* sp. (Norway) were used as hosts and cultivated on 1 per cent dextrose and 1 per cent proteose-peptone agar media (2 per cent agar). In the proteose-peptone medium, *D. cornuta* almost completely covered the bacterioid colony (25 mm. in diam.) of *M. genevensis* while on the semi-bacterioid colony (50–52 mm. in diam.) of *Mucor* sp. (Norway), it produced a limited mass of fertile hyphae which spread over an area approximately 12–15 × 7–8 mm.

In contrast, *D. cornuta* failed to produce any fertile hyphae on *Mucor* sp. or *M. genevensis* (Mutant) when these hosts were cultured on the 1 per cent dextrose medium. It is interesting to note that the growth of these two species of *Mucor* on the above media was markedly different, for on the proteose-peptone medium, *M. genevensis* (Mutant) formed a dense, bacterioid colony, cream colored, 25 mm. in diam., while on the dextrose medium it produced an open, colorless, plumose type of growth which was about 15 mm. in diam., and *Mucor* sp. (Norway) on the dextrose medium produced an open smoke-gray growth which covered the entire surface of the substratum (66 mm. in diam.) while on the proteose-peptone, it formed a more compact, bacterioid colony (50–52 mm. in diam.).

#### CONSTANCY OF IMMUNITY OF MORTIERELLA ECHINULATA TO DISPIRA CORNUTA

Although most of the Mucorales species tested in this investigation were found to be hosts of *D. cornuta*, *Mortierella echinulata* and several unidentified species of *Mortierella*, and *Piptocephalis* sp. were found to be immune to this parasite. An attempt to alter the immunity of *M. echinulata* was made by growing it on a proteose-peptone medium which had been demonstrated in a previous experiment to affect the susceptibility of *Mucor* sp. and *Mortierella* sp. to *D. cornuta*. This medium consisted of 8 grams of peptone, twenty grams of agar, and one liter of distilled water. To this medium transfers from pure cultures of *Mortierella echinulata* were made after which the host was inoculated with spores and mycelium from pure cultures of *D. cornuta*.

These cultures were stored in a place favorable for the growth of both organisms and kept under observation for several weeks. During this period *M. echinulata* grew so vigorously it covered the entire surface of the medium in the Petri dishes yet *D. cornuta* never developed on this organism under these conditions. Since this experiment was repeated several times, and *D. cornuta* was never observed growing in these cultures, it seems justifiable to conclude that the immunity of *Mortierella echinulata* sp. was not affected by cultivating the organism on a type of medium which had been previously demonstrated to favor the susceptibility of other organisms to the parasite under discussion.

Since all attempts to break down the resistance of the host failed, an effort was made to increase the pathogenicity of *D. cornuta* by growing this parasite first on a susceptible species, *Mortierella* I, then on a somewhat resistant form, *Mortierella* III, and finally transferring it to the immune *M. echinulata*. Following this procedure, numerous attempts were made to infect *M. echinulata* with *D. cornuta* but they were unsuccessful. The pathogenicity of the parasite did not increase as a result of development on such "bridging" hosts.

#### DISCUSSION

This investigation on the parasitism of *Dispira cornuta* van Tiegh. brings out some points of interest in connection with its identity, its morphology, and its parasitism.

The identity of *D. cornuta* has been confused considerably since it was first described. Although the fungus had been reported but four times in the past, it had been listed three times under the following different names, *D. cornuta*, *D. americana*, and *D. circinata*. As previously pointed out, this confusion in the identification of the fungus had resulted mainly from erroneous descriptions and inaccurate illustrations of the fungus, and from the failure to realize that it is very plastic in culture under different external conditions. For example, if van Tieghem had illustrated the *D. cornuta* correctly, Thaxter probably would not have considered the American fungus to be a distinct species. Still it must be admitted that if Thaxter had examined authentic material of van Tieghem's fungus, he undoubtedly would have

seen that he and van Tieghem had the same organism. Yet for a fungus with such distinct morphological characteristics, accurate drawings should be sufficient for its identification. Bainier's explanation that van Tieghem studied the fungus in earlier stages of development than those seen by Thaxter is unreasonable because an examination of Thaxter's plate for *D. americana* shows that he examined the fungus in all stages of development, from the formation of the primary sterigmata to the maturation of the spores. Similarly Elliott, in the case of *D. circinata*, failed to observe that this fungus is very plastic in culture and produces a variable number of branches. At the time she described *D. circinata*, Elliott apparently was unaware of Bainier's emended description of *D. cornuta* because she compared *D. circinata* with *D. cornuta* as described and illustrated by van Tieghem and pointed out the differences between these supposedly different species. Thus after considering the characters upon which the different species of *Dispira* were delimited and after studying the fungus in culture it is certain that there is only one species of *Dispira* known, and that is *D. cornuta*.

The exact position of this fungus in classification has never been definitely decided and it has been included among the Phycomycetes, Ascomycetes, and Fungi Imperfecti. To date, this fungus has never been connected with a sexual stage, although Thaxter (18) reported finding zygosporcs which he later decided were the feeding cells of *Parasitella* on *Mucor*. However, Thaxter (18) believed that *D. cornuta* was a member of the Mucorales because of its general habit, the peculiarities of the sporophores, the coherence of the spore mass when ripe, and the peculiarities of the septa which are common to both *Dimargaris* and *Dispira*. Van Tieghem (21), on the contrary, believed this aberrant group of fungi, *Dispira*, *Dimargaris*, *Coemansia*, *Kickxella*, and *Martensella*, were members of the Ascomycetes because he claimed that he had found perithecia in his *Kickxella* cultures. However, Thaxter, who had cultivated several of these forms in pure culture for many years, never found any ascigerous stages whatever. Likewise the writer has never observed *Dispira cornuta* or *Coemansia reversa* producing a sexual stage in culture.

under different conditions, either when they were cultivated alone or when they were in the presence of various hosts. This of course is negative evidence and it may be that the necessary conditions for production of the sexual stage were not provided. Yet even though lacking such definite proof as zygosporcs, there are certain points which suggest that *Dispira* probably is a member of the Mucorales. For example, as illustrated by Thaxter (18), although he did not emphasize the fact in his early work on the same fungus, the asexual spores are sporangiospores instead of conidia because an outer wall can be detected around the outside of the spores, making, instead of a conidial chain, a reduced linear sporangium similar to those of *Piptocephalis*, *Syncephalis* and *Syncephalastrum*. Similarly the sporangia are borne on a head as in *Piptocephalis*, although in *Dispira* this is complicated by the presence of primary and secondary sterigmata. In addition to these points, the fertile hyphae of *Dispira* are similar in form to those of *Piptocephalis*, as in both cases they are upright, arise from a creeping mycelium which is parasitic on members of the Mucorales and are septate from the earliest stages of development. The writer has observed that septa are formed soon after germination in the germ tube of both organisms, in contrast to most members of the Mucorales, which are coenocytic or at least not septate until fully mature. Yet *D. cornuta* cannot be excluded from the Mucorales on this basis. Furthermore, the presence of *D. cornuta* in the same habitat as *Piptocephalis* and other members of the Mucorales, its early appearance in culture, on such substrata, and the fact that *D. cornuta* like species of *Piptocephalis* is parasitic only on fungi which are members of the Mucorales suggest that *D. cornuta* belongs in the same order.

Like *D. cornuta*, *Coemansia reversa* has been considered in the past as one of the Phycomycetes, Ascomycetes, and Fungi Imperfecti. Therefore, it was tested as a host for both *Dispira cornuta* and *Piptocephalis* sp. because these fungi so far have been found to be parasitic only on members of the Mucorales. As shown in Table II, *C. reversa* was not a host for either *D. cornuta* or *Piptocephalis* sp. However, these results neither prove nor disprove that *C. reversa* belongs in the Mucorales since there are

a very few members of the order, such as species of *Mortierella*, which are not hosts for *D. cornuta* or *Piptocephalis* sp. On the other hand, if *C. reversa* were a host for *D. cornuta* and *Piptocephalis* sp., this positive evidence would seem sufficient proof that *C. reversa* belongs in the order since, as previously mentioned, only members of the Mucorales have been demonstrated to be hosts for these parasites. Although the negative evidence is of course not conclusive, it seems to indicate that *C. reversa* is probably not one of the Mucorales.

The host range of *D. cornuta* is interesting since, as has been demonstrated in this paper, it includes only members of the Mucorales, an order to which the fungus itself in all probability belongs. In its host range, the writer has found members of the Mucoraceae, Thamnidiaeae, Pilobolaceae, Mortierellaceae, Choanephoraceae, Chaetocladiaceae, and Cephalidaceae even though previously only species of the genus *Mucor* had been found to be hosts. This host range is far more extensive than those reported by various authors for other parasitic species of the Mucorales, comprising, for example, more genera of Mucorales than the host ranges reported by Burgeff (6, 7) for *Parasitella simplex* (plus and minus strains) and *Chaetocladium* sp. Burgeff found the host ranges of these two fungi included only *Rhizopus*, *Zygorhynchus*, *Sporodinia*, *Thamnidium*, *Chaetostylum*, *Pilaira*, and *Choanephora*, while he observed that no reaction occurred between *Parasitella simplex* or *Chaetocladium* sp. and the following genera: *Phycomyces*, *Mucor Ramannianus*, *Pilobolus*, *Thamnidium*, *Cunninghamella*, and *Syncephalastrum*. As shown in Table II, *D. cornuta* has been found to attack all of the above-mentioned genera with the exception of *Phycomyces*, *Pilaira*, and *Mucor Ramannianus* which unfortunately could not be secured for tests as potential hosts. Similarly the host range of *D. cornuta* includes a greater number of the Mucorales than that of any species of *Piptocephalis* reported in the researches of Brefeld (5), van Tieghem (21), Matruchot (15), or of others as listed in different host indices, since they include no species of the Mortierellaceae, Choanephoraceae, and Cephalidaceae.

It is significant that *D. cornuta* should be limited in its parasitism to the members of the Mucorales even though it is not

parasitic on every member of this order. One would expect that a parasite on one fungus could parasitize almost any other fungus because of the simple structure of these organisms as compared to the complex structure of the higher plants. Probably the cell walls of the immune forms cannot be penetrated by the feeding hyphae of this fungus because, as shown in the case of susceptible forms, the fungus is unable to penetrate the cell walls when they are mature. However, Howard (10) in his studies on the feeding habits of *Physarum polycephalum* found that this organism would not feed on various species of Mucorales but it was able to utilize different members of the Ascomycetes and Basidiomycetes as a source of food, thus demonstrating a difference between the Mucorales and other fungi in their composition. It is interesting that the same phenomena of immunity, resistance, and susceptibility should be encountered in these lower forms of plant life as in the higher because perhaps too generally it is assumed from their simplicity of form that their physiological processes are primitive. Further, this restriction of certain hosts suggests a relationship between *Dispira* and *Piptocephalis* since both organisms are restricted in their parasitism to the same group of organisms and both have been considered to be obligate parasites on this group of which *Piptocephalis* has been demonstrated by its zygospores definitely to be a member.

It is of interest, furthermore, that the composition of the media had such a decided influence on the susceptibility of certain hosts to *D. cornuta*. Perhaps these hosts when cultivated on such media form products which either are unsuitable for the food requirements of the parasite or are antagonistic to its growth. However, it is possible that the change in osmotic concentration or hydrogen-ion concentration may have some effect even though Hurd (11) demonstrated that there was no correlation between hydrogen-ion concentration of the expressed juice of wheat plants and the resistance or susceptibility of wheat varieties to rust, and Hursh (12) found no correlation between the amount of solids in the sap of wheat plants and their resistance to rust although he did find differences in the sugar content affected the resistance of some varieties. In contrast to these results,

certain investigators have found that the sugar content of wheat plants or their nutrition by sugars increased the susceptibility to certain parasites. Pantanelli (16) for example found that the organs of the wheat plant most susceptible to rust are those richest in sugars, acids, and soluble compounds of phosphorus and nitrogen. Similarly Trelease and Trelease (20) demonstrated in the case of wheat and mildew (*Erysiphe*) that mineral nutrition of the wheat leaves which were grown in test tubes was not a limiting factor in the ability of the leaves to support mildew. However, they found that dextrose, levulose, sucrose and melizitose were most effective in increasing the susceptibility of the leaves to mildew while xylose, galactose, maltose, lactose, and glycerine were less effective and arabinose, rhamnose, mannose, and the non-sugars, starch, dextrin, and inulin or alcohol and glycerine were least effective. On the other hand, Thomas and Muller (19) observed that sugars such as dextrose (3 per cent), galactose (1 per cent) and saccharose (3 per cent) applied to plants through the roots decreased the quantity of infection; but whether these results are due to assimilation of sugars, changes in soil flora, or soil toxicity, was not determined. This brief review of the previous investigations on the relation of sugars to susceptibility and resistance shows divergent results because the fungi used in these experiments differ in their food requirements and the different host plants elaborate various foodstuffs. Although the susceptibility and resistance of higher plants have been changed by nutrition, the present work of *Dispira* seems to be the first instance in which the susceptibility and resistance of a fungus as host have been altered by such means.

It is of interest also that the age of the host hyphae was observed to be important in the relation between *Dispira* and its host for a similar situation has been found in the case of flowering plant hosts. Leach (13), for example, showed that *Colletotrichum Lindemuthianum* produced smaller lesions on old plants than on young plants. Similarly Anderson (1), and Walker and Jones (22) observed that the onion is susceptible to smut (*Urocystis Cepulae*) in the seedling stage, but it is resistant after it passes the cotyledonous stage. Also other investigators have reported the same phenomena in the relationship between various other

hosts and parasites but this is probably the first report of a similar phenomenon in the case of a fungus parasitizing another fungus.

The sex of the host has been claimed by some investigators to affect the relationship between host and parasite. For example, Burgeff (6) reported that a plus strain of *Parasitella* would parasitize only a minus strain of *Absidia* and vice versa. He suggested as a result of his findings that parasitism might have resulted from an imperfect sex reaction. However, in the case of *Dispira*, the writer observed that this fungus parasitized equally the plus and minus strains of *Rhizopus nigricans*, *Absidia glauca*, *Cunninghamella echinulata*, and *Blakeslea trispora*. Similarly, Satina and Blakeslee (17), using a large number of different physiological strains of plus and minus of *Parasitella* and *Absidia*, found that the formation of galls occurred not only when the host and parasite were of opposite sex but also when they were of the same sex. But they observed that this formation of galls occurred more frequently between the opposite sexes.

This study of the parasitism in *Dispira tornuta* is only a small beginning in a field of investigation that holds many opportunities and potentialities. The writer hopes to carry this work further in the future, but even now from this initial step it is clear that this group of relatively simple fungi offers valuable and promising material for the effective investigation of certain significant problems of parasitism.

#### SUMMARY

1. *Dispira cornuta* van Tiegh., the fungus used in this study, is one of the less known, so-called "conidial" species of the Mucorales. It was found initially by the author associated with an unidentified species of *Mucor* growing on hog dung from Waltham, Mass.

2. The fungus previously had been reported only twice from France, once from England, and once from North America, and hence had been considered a rare species. The writer has collected it six times since learning the requirements of the fungus.

3: Three species, *Dispira cornuta*, *D. americana* and *D. circinata*, have been reported but an intensive study of the writer's

fungus on its natural hosts and in pure culture for over two years has shown the range of variation in structural features to include the various characteristics emphasized as distinctive in the previous descriptions and illustrations. It seems justifiable to assume, therefore, that the three species are in reality reducible to one.

4. *D. cornuta* was found to be parasitic on members of the Mucorales only although a long list of fungi representing not only other orders of the Phycomycetes, but also Myxomycetes, Ascomycetes, Basidiomycetes, and Fungi Imperfecti were tested as possible hosts.

5. Within the Mucorales, the fungus was found to parasitize representatives of all genera tested although it was not equally parasitic on the different species used.

6. The fungus is equally parasitic on the male (minus) and the female (plus) strains of such heterothallic fungi as *Rhizopus nigricans*, *Cunninghamella echinulata*, *Blakeslea trispora*, and *Absidia glauca*.

7. In comparison with other parasitic members of the Mucorales, *D. cornuta* has a more extensive range of hosts than either *Parasitella* or *Chaetocladium* and one which is fully equal to that of the several species of *Piptocephalis*.

8. Neither *D. cornuta* nor *Piptocephalis* were parasitic on *Coemansia reversa*, a little-known conidial fungus which by some has been considered to be a member of the Muçorales because of its aberrant methods of growth and of conidial formation, and by others a member of the Ascomycetes.

9. In the case of the genus *Mortierella*, it was found that *D. cornuta* readily attacked *Mortierella* I (an undetermined species of *Mortierella* seemingly new); was only weakly parasitic on *Mortierella* III, and apparently utterly unable to parasitize four other species of this genus.

10. On species of *Mucor* and on *Mortierella* I known to be susceptible to it, *Dispira* was never able to develop when the hosts were grown on agar media containing dextrose, but grew luxuriantly when the hosts were grown on proteose-peptone agar, an instance of the alteration of the susceptibility and resistance of a host through its nutrition, not as yet reported among the fungi.

11. The morphological details of the relation of the parasite and the host were studied and it was found that in the presence of suitable hosts the spores of *D. cornuta* swelled, produced one or more germ tubes which elongated, became septate, and branched, ultimately reaching the hyphae of the host.

12. If the hyphae of the host were young, thin-walled, and growing actively the parasite penetrated, establishing haustoria within. These developed, only in the vegetative, creeping hyphae of the host, never forming in sporangia, sporangiophores or in the aerial hyphae.

13. When the vegetative hyphae of such hosts as *Sporodinia grandis* became mature, the hyphae of the parasite were no longer able to penetrate and establish haustoria within them.

14. The general significance of some of the aspects of the parasitism of *D. cornuta* is considered in relation to other pertinent cases of parasitism reported in the literature, and its importance is discussed.

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# A SPECIES OF SORODISCUS ON HETERANTHERA<sup>1</sup>

CLIFFORD C. WERNHAM<sup>2</sup>

(WITH PLATES 17, 18 AND 2 TEXT FIGURES)

The fungus discussed in this paper was first collected in Lake Champlain in August 1929 by Doctor W. C. Muenscher of the Department of Botany of Cornell University. While engaged in a biological survey for the New York State Conservation Department he observed characteristic galls on the lower portions of the stem of *Heteranthera dubia* (Jacq.) MacM., and in these found the organism constantly present. Specimens were submitted to Doctor H. M. Fitzpatrick for identification, and, because of the flat disc-like aspect of many of the spore aggregations, the organism was referred by him tentatively to *Sorodiscus* of the Plasmodiophoraceæ. An American collection of this genus was of sufficient interest to warrant a more complete study, and the investigation herein presented was begun.

Additional collections of the organism have been made by Doctor Muenscher and the writer in various lakes and streams in or bordering New York State. The localities are marked on the accompanying map (TEXT FIG. 1). Observations to date indicate that the distribution of the fungus may be coextensive with that of the host. As *Heteranthera dubia* occurs on mud bottoms in water of a depth of one or two meters it is usual for the plants to be completely submerged. During low water levels they are sometimes found exposed on mud flats. The galls caused by the presence of the fungus are usually hypertrophied adventitious roots, and these are found considerably below the surface of the water at the nodes of the stem (FIG. 2). Galls occur also on the true roots, though these were not observed until 1933 when

<sup>1</sup> Presented before the Mycological Society of America at its second annual meeting at Boston, Massachusetts, December 29, 1933.

<sup>2</sup> The writer wishes to acknowledge his indebtedness to Professor H. M. Fitzpatrick, under whose continued helpful supervision this investigation was prosecuted.

collections were made of plants growing on a submerged cinder fill at the southern end of Seneca Lake (FIG. 1).

The type species of *Sorodiscus*, *S. Callitrichis* Lagerheim & Winge, occurs in Europe on at least two species of *Callitriche*.

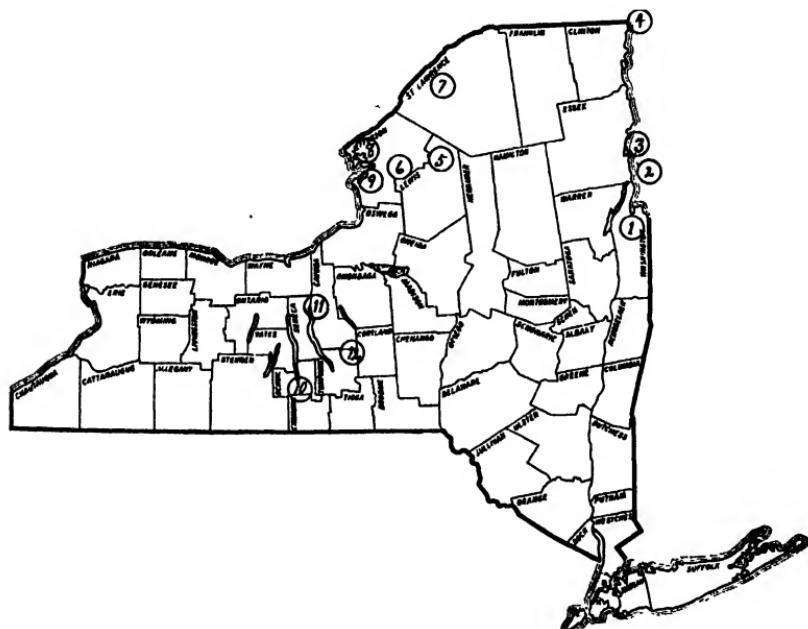


FIG. 1. Map of New York State with indication of localities in which *Sorodiscus Heterantherae* has been collected. 1, South Bay, N. Y.; 2, Dresden, N. Y.; 3, West Haven, Vermont; 4, Missisquoi Bay, Quebec; 5, Bonaparte Lake; 6, Perch Lake; 7, Black Lake; 8, Mud Creek; 9, Chaumont River; 10, Seneca Lake; 11, Cayuga Lake; 12, Dryden Lake.

One of these, *C. autumnalis* L., occurs commonly in New York associated with *Heteranthera dubia*. It is a significant fact that, although plants of this species have been examined at every opportunity, no galls have been found on them by us in any case.

#### HISTORICAL.

The Plasmodiophoraceae embrace a rather small group of apparently primitive organisms, living as endoparasites in the cells of higher plants.

In most cases the symptoms are expressed in hypertrophied tissues. . The close relationship of the various genera included

is adequately shown by a striking similarity of their life cycles. A brief description, at this point, of the typical life cycle will serve to prepare the reader for consideration of the material presented in this paper.

The thallus at first is an intracellular, uninucleate, amoeboid protoplast (myxamoeba) which increases in size as it absorbs nutrients from the cell. The nuclear divisions which occur during the growth period are peculiar to these organisms. The term "cruciform division" is commonly applied since at metaphase the persisting nucleolus divides at right angles to the equatorial plate and the two, if viewed from the side, give the appearance of a cross. At telophase the aspect is that of a "double anchor."

As the myxamoeba increases in size and in number of nuclei it may or may not fragment into daughter myxamoebae. When growth terminates, meiosis takes place. The nucleolus disappears during the prophase of the first division as in higher plants. Following meiosis the myxamoeba is partitioned into uninucleate portions by cleavage planes. Each portion envelops itself in a wall and assumes a more or less spherical form. These spherical bodies, after the formation of the wall, are called spores. The spores may remain attached to one another in groups of definite conformation, or they may fall apart and lie free in the host cell. After passing through a period of rest the spore germinates by one or more swarmspores or myxamoebae. Whether these reinfect the host directly or first fuse to form zygotes, is in dispute.

The type of spore aggregation has afforded bases for recognition of several genera. Cook (3), in his monographic study, separates these genera into two groups: those in which the myxamoeba forms spores only, and those in which it gives rise to both spores and zoosporangia. The first group embraces five genera: *Plasmodiophora*, in which the spores at maturity lie free in the host cell, *Sorosphaera*, in which they constitute a hollow sphere, *Sorodiscus*, in which they lie in two closely adherent layers forming a solid flattened ball or discus, *Spongospora*, in which the spore-ball is a more or less solid sphere transversed by fissures, and *Tetramyxa*, in which the spores adhere in tetrads. *Soro-*

*sphaera* and *Sorodiscus*, according to Cook, are further differentiated from *Spongospora* and *Tetramyxa* by the presence in the former genera of a common soral membrane about the spore aggregation. The second group includes only the genus *Ligniera*, and in it the spores are aggregated in indefinite fashion.

The literature on *Sorodiscus* is limited and not without controversial features. The first published paper is that of Kareltschikoff and Rosanoff (5).. They describe and figure galls on *Callitricha autumnalis* containing flat plate-like bodies of constant thickness, consisting of very small polygonal cells arranged in two layers. Kareltschikoff reports on these as microchemical structures, while Rosanoff figures a mycelial organism for which the bodies appear as the spore form. Maire and Tison (7) believed the mycelium of Rosanoff to have no connection with the spores and state that the organism is probably plasmodiophoraceous. It seems indeed highly likely that Kareltschikoff and Rosanoff actually collected in 1870 at St. Petersburg on *Callitricha autumnalis* the same fungus that Lagerheim (according to Winge) found later in 1893 and 1900 on *C. vernalis* in Norway. Subsequent collections on *Callitricha autumnalis* were not reported until 1907, when Ostenfeld (Anonymous 1) encountered the organism in two localities in Sweden. Lagerheim, on the basis of his own studies, regarded the organism as a myctozoan, and because of the characteristic arrangement of its spores in flat discs erected the new genus *Sorodiscus* to include it, naming the species *S. Callitrichis*. Lagerheim's point of view remained unsubstantiated until Winge (10) presented a cytological study of the organism and gave us for the first time a technical diagnosis of the genus and species. He mentions the great similarity between *Sorodiscus* and *Sorosphaera*, but maintains that recognition of the former is justifiable on the basis of the arrangement of the spores at maturity, i.e., in spore-cakes, two layers thick. Winge does not state that a common membrane exists about the spore-cake but says, "At the full maturity of the spore aggregation the spore wall divides into two layers, of which the outer one merges into that of the neighboring spores so that it gives one the impression of the spores being deposited in a common substance."

For fourteen years following the appearance of Winge's paper no other contribution on the genus was made. Karling (6) gives an inadequate description of a fungus found causing cell hypertrophy in *Chara contraria* and *C. delicatula* at Oberlin, Ohio. As described by him, a sporangiosorus arises from a multinucleate naked protoplast and has the appearance of a flattened disc consisting of a number of definitely operculate cells. Unfortunately his material was lost before a cytological study was completed and the organism remained unnamed until Cook (3) saw fit to include it as a new species of *Sorodiscus*, *S. Karlingii*. Meanwhile, on the basis of Winge's work, Fitzpatrick (4) had incorporated *Sorodiscus Callitrichis* in the Plasmodiophoraceae of the Chytridiales as a monotypic genus.

Cook (2), from specimens sent him from South Africa by Ethel M. Doidge, described in 1931 *Sorodiscus radiciculus* as causing tumors on roots of an orchard weed, *Gynandropsis pentaphylla*. The smaller size of the spore-balls and individual spores, combined with the wide difference in host relationship, is considered by Cook as providing sufficient basis for separating this fungus from the European species. He states that the spore-ball "is enclosed in a delicate membrane surrounding the wall of the spores." There is no inference that this delicate membrane is the result of the fusing of the outer walls as mentioned by Winge.

Palm and Burke (9) present striking data on a plasmodiophoraceous fungus infecting *Veronica americana*. So variable are the spore aggregations of this species that the authors hesitate to place it in any one of the genera *Sorosphaera*, *Spongospora*, *Ligniera*, or *Sorodiscus*. Because of this situation the authors lament the unsatisfactory taxonomic condition in the Plasmodiophoraceae and suggest that several of the genera be merged.

Basing his conceptions on *Sorodiscus Callitrichis* and *S. radiciculus*, Cook (3) rigidly delimits the genus *Sorodiscus*. He stresses as of generic significance the arrangement of spores in a flat spore-cake of two layers, the shape of the spores, and the common membrane enclosing the spore-cake. His readiness to incorporate the organism described by Karling indicates, however, that spore arrangement is actually in his mind the fundamental

character used in delimiting genera. His point of view is thus wholly at variance with that of Palm and Burke.

In his treatment of the Plasmodiophorales Cook (3) apparently overlooked a paper published in 1930 by Ostenfeld and Petersen (8). In it these authors erected the genus *Membranosorus* and the species *M. Heterantherae* on a species found in 1924 at Lake of the Woods, Ontario, Canada. This fungus causes hypertrophy of the adventitious roots at the nodes of the stem of *Heteranthera dubia*. These roots, when young, are whitish, but when the contained spores of the fungus ripen they grow dark in color. The spores of the fungus arise from a multinucleate myxamoeba within the host cell and as seen in section seem to adhere in a single layer lining the inner surface of its wall. It is this feature of the fungus which led the authors to coin the word *Membranosorus*. So constant was this character in the scanty material which they examined that they felt justified in creating a new genus. The fact that the host is identical with the one under consideration in this investigation, and that the fungus invades similar tissues raises the question of the identity of their organism with our own.

#### LIFE CYCLE AND MORPHOLOGY

The galls on the adventitious roots seem to proliferate from the point of origin of the root at the node of the host. They are dark olive brown to black in color (FIG. 2) and at their base vary considerably in size, measuring 0.5–3.0 cm. in thickness. Protruding from the body of the gall are a number of finger-like projections, 0.5–1.5 cm. in length. The galls on the true roots are somewhat lighter in color and generally smaller. At maturity they have the same contour as those on the adventitious roots. The size of the gall appears to bear no relation whatever to the stage of maturity of the fungus. Though in a few of the galls only myxamoebae are found and in others only mature sori, most of them contain both stages of the organism.

Whether the zoospores, which cause infection, are haploid or diploid has not been determined. Sections cut 7  $\mu$  in thickness from the tips of the galls and stained either with iron-alum haematoxylin or with aceto-carmine and fast green give satis-

factory material for the study of the myxamoebae.<sup>3</sup> In our material these usually range from 8  $\mu$  to 28  $\mu$  along the greater axis. Longer myxamoebae reaching 70  $\mu$  in length sometimes partially line the wall of the host cell and form a band about its protoplast (FIG. 4). The aspect in section is then that which would be given if the host cell wall were completely lined as claimed for *Membranosorus*. Though the host cell often contains only a single myxamoeba, it is not unusual for several to be present.

The nuclei of the myxamoeba are rather evenly spaced throughout the cytoplasm, the number varying with the size of the protoplast. The myxamoeba, during the vegetative period, is characterized by large nuclei (FIG. 5) slightly more than 3  $\mu$  in diameter, which exhibit a cruciform appearance at metaphase (FIG. 6) or the aspect of a double anchor at telophase. No centrosomes or astral rays were observed in these division figures in our preparations. The nuclei typical of myxamoebae about to undergo meiosis (FIG. 7) are smaller, being approximately 2.5  $\mu$  in diameter. The transition from the large vegetative nuclei to these smaller nuclei was not followed. Prophases, metaphases, anaphases, and telophases of the first meiotic mitosis were plentiful in our preparations and easily recognizable. During the prophase of this mitosis the nucleolus disappears completely and a normal metaphase follows. Centrosomes have not been seen, but astral rays are evident. The number of chromosome pairs was not determined with accuracy, but is probably four, five, or six. About these nuclei at metaphase a differentiation of cytoplasm suggestive of that in spore mother cells was noted (FIG. 9). The interphase nuclei (FIG. 8) are small (approximately 2  $\mu$  in diameter) and can be distinguished by their smaller size from the nuclei of the preceding growth stages. The second meiotic mitosis has been observed, but, on account of the extremely minute figures, has not been satisfactorily studied. The metaphase figures are not cruciform, nor is the evidence of subsequent spore mother cell division clear. It is realized that imperfection in staining technique may be responsible for this

<sup>3</sup> Miss S. M. Allen, experimenting with this material in the microtechnique course, in the Department of Botany, obtained the best preparation using Randolph's crystal violet method [Stain Technology 10: in press. 1935].

lack of evidence. Since all the nuclei in the myxamoeba divide simultaneously they are always of the same stage of the life cycle. Nuclei representing more than one type are therefore never found together in a single myxamoeba.

Differentiation in the cytoplasm follows the last mitosis closely, and soon cleavage planes appear between the nuclei cutting the protoplast into uninucleate portions (FIG. 10). These round up and assume definite walls which gradually thicken. A sorus of globose or ovoid spores lacking a common soral envelope thus results (FIG. 11-18). The total number of spores resulting from partition of the myxamoeba varies greatly, but is believed to be always some multiple of four.

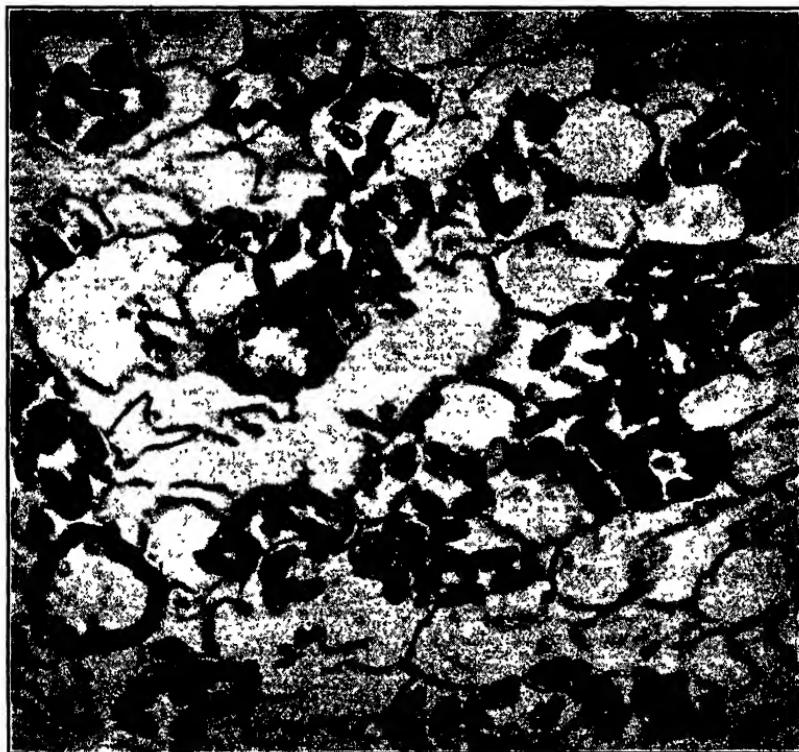


FIG. 2. Spore aggregations of *Sorodiscus Heterantherae* in the cells of the host,  
 $\times 360$ .

The sori vary greatly with respect to the arrangement of the spores in layers (TEXT FIG. 2). Single layered sori are common

(FIG. 15, 16), but sori with two complete layers are exceptional. This latter type of arrangement is not infrequently approximated, however, by sori of the conformation illustrated in figures 14 and 18. The majority of the sori observed display an arrangement in which the greater number of spores are aligned in a single layer on which are superimposed the remaining spores of the myxamoeboid complement (FIG. 11, 12, 17). Occasionally sori were observed in which orientation into definite layers was wholly lacking (FIG. 13), the spores being aggregated merely in an irregular mass. The mature spores have thick buffy brown (Ridgeway) walls and are 3.5–4.5  $\mu$  in diameter. Occasionally slightly irregular thickening of the otherwise smooth wall has been observed, but an apical ring or collar is noticeably absent.

Time has not permitted an attempt to germinate the spores under carefully controlled conditions. During the course of the investigation another correlated problem presented itself. Large multisporous sacs, which are apparently sporangia, were sometimes observed in the outer cells of the gall tissue. Whether these represent a phase in the life cycle or merely result from invasion by another fungus is not certainly known. Proof of existence of sporangial stage in the life cycle can be furnished beyond doubt only by growing the host in pure culture and by seeding the culture with spores of the organism. In the light of our present knowledge there is not sufficient reason for regarding these sacs as constituting a sporangial stage of *Sorodiscus*.

#### DISCUSSION

The study of the life cycle of the organism in *Heteranthera*, while not complete, has yielded sufficient data to warrant inclusion of the species in the Plasmodiophoraceae. The naked protoplast, with simultaneous nuclear divisions of the cruciform type succeeded by normal meiosis and subsequent formation of groups of uninucleate spores, presents the generally recognized cytological characteristics of the family. In addition, the organism causes marked hypertrophy of the infected tissues.

*Sorodiscus* was erected to include members of the family in which the spores are arranged in flat discs. The previously described species of the genus, without exception, have discs

of two spore layers in thickness. The species under consideration varies from this arrangement in that the discs are not composed consistently of two layers. Indeed so numerous are the deviates from the two-layer arrangement that these cannot be ignored. Gross dissection reveals these variants in such number that their presence in prepared slides cannot be explained as due to vagaries of microtechnique. Palm and Burke (9) have encountered a similar situation in *Sorosphaera Veronicae* on *Veronica americana*.

The frequent occurrence in our organism of spores in one-layered ribbons or bands (FIG. 3) has led us to believe that it is closely related to, if not identical with, *Membranosorus Heterantherae* Ostenfeld & Petersen. While not a single case of a host cell lined with a complete layer of spores, as described by them, has been observed, the condition is approached in many instances. The spores agree closely in size in the two cases. Moreover, the same tissues of identical hosts are involved in the two species with strikingly similar resultant aspects. Since these authors state that their material was extremely scanty, it is not unlikely that their species represents merely a morphological variation of our fungus.

According to Winge the spores of *Sorodiscus Callitrichis* appear to be imbedded in a matrix due to the fusion of their outer wall, while Cook states that the spore-cake of *S. radiciculus* is enclosed in a delicate soral membrane. Neither of these conditions exists in our material; nor is there present an apical ring or collar such as that figured for *S. Karlingii*. Occasionally there is some irregularity in the thickness of the wall, but in general the spores are wholly smooth. They are spherical to ovoid in shape and lack a definite axis.

Though we have not had available for comparative study material of the type species, *S. Callitrichis*, the differences evident from the description are regarded as of relative unimportance, and as insufficient to warrant exclusion of our species from the genus. As only slight broadening of the concept of the genus is necessary to include it, we propose that it be incorporated as a new species and submit the following diagnosis.

**Sorodiscus Heterantherae sp. nov.**

Mature thallus a multinucleate flattened naked protoplast, various in shape and size, sometimes ellipsoidal and as little as 8  $\mu$  in diameter, but more typically a disc or ribbon which not infrequently encircles the host protoplast as a definite band which may reach 70  $\mu$  in length; vegetative mitoses cruciform; meiosis occurring in the mature thallus immediately preceding cytoplasmic cleavage and spore delimitation; a soral membrane not formed; spore aggregations extremely various in size, shape, and with respect to the arrangement of the spores in layers, but total number of spores in the aggregation believed to be always some multiple of four; single-layered sori common; sori with two complete layers exceptional; intermediate types abundant; some sori failing to show differentiation into layers; others definitely ribbon-like or plate-like and often tending to line the inner surface of the host cell wall more or less completely; individual spores at maturity globose to ovoid, 3.5–4.5  $\mu$  in diameter, buffy-brown (Ridgeway), and thick-walled; spore wall smooth, not always of uniform thickness, lacking an apical ring, collar or operculum, varying in thickness from 0.6 to 1.0  $\mu$ ; method of spore germination unknown; swarmspores not observed, and phase of life cycle outside of host not studied.

Parasitic in *Heteranthera dubia* (Jacq.) MacM. causing the formation of prominent dark olive brown to black galls, 0.5–3.0 cm. in diameter, on the true and adventitious roots; galls characterized by finger-like projections, 0.5–1.5 cm. in length, and containing the organism within their cells.

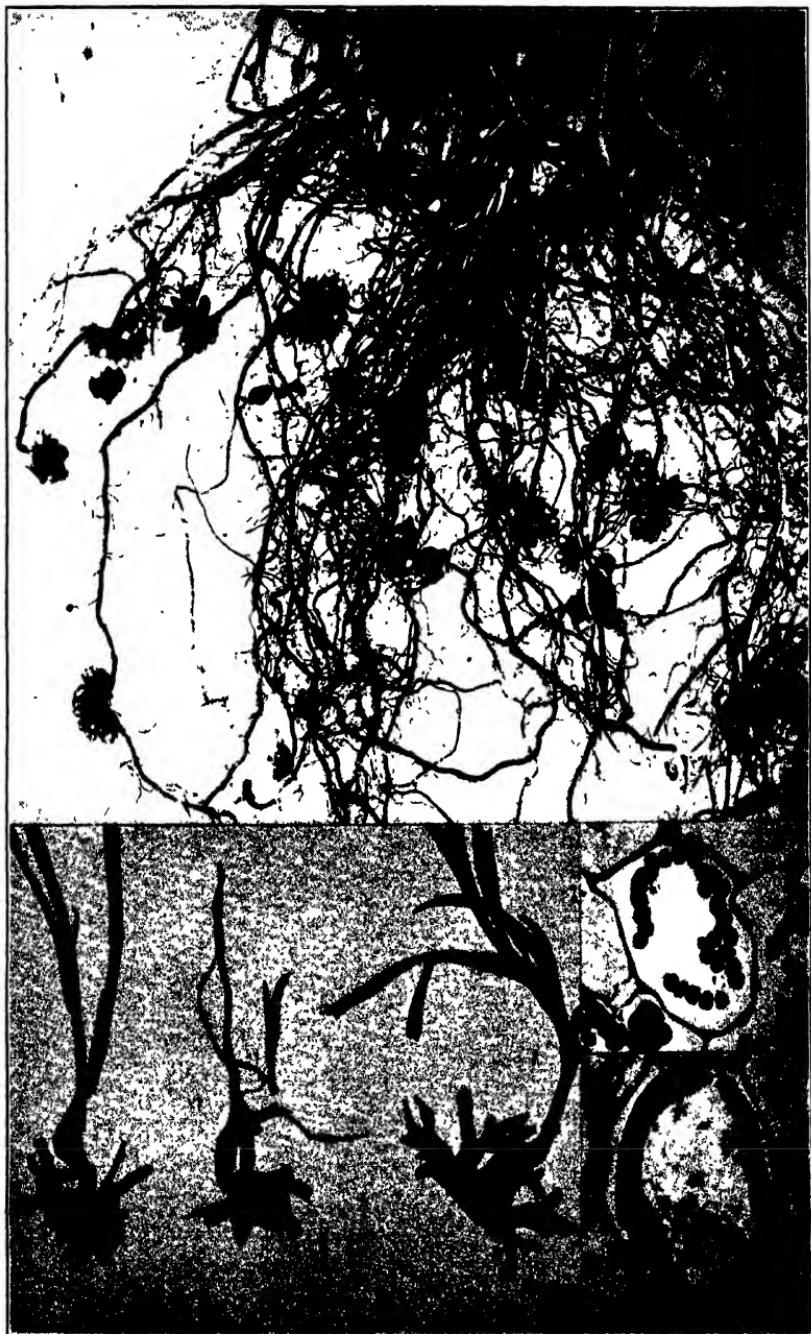
Occurring in various lakes and streams of the St. Lawrence basin, and perhaps elsewhere in northeastern North America.

Type material deposited at Cornell University, Harvard University, The New York Botanical Garden, Bureau of Plant Industry, and the Royal Botanic Gardens at Kew.

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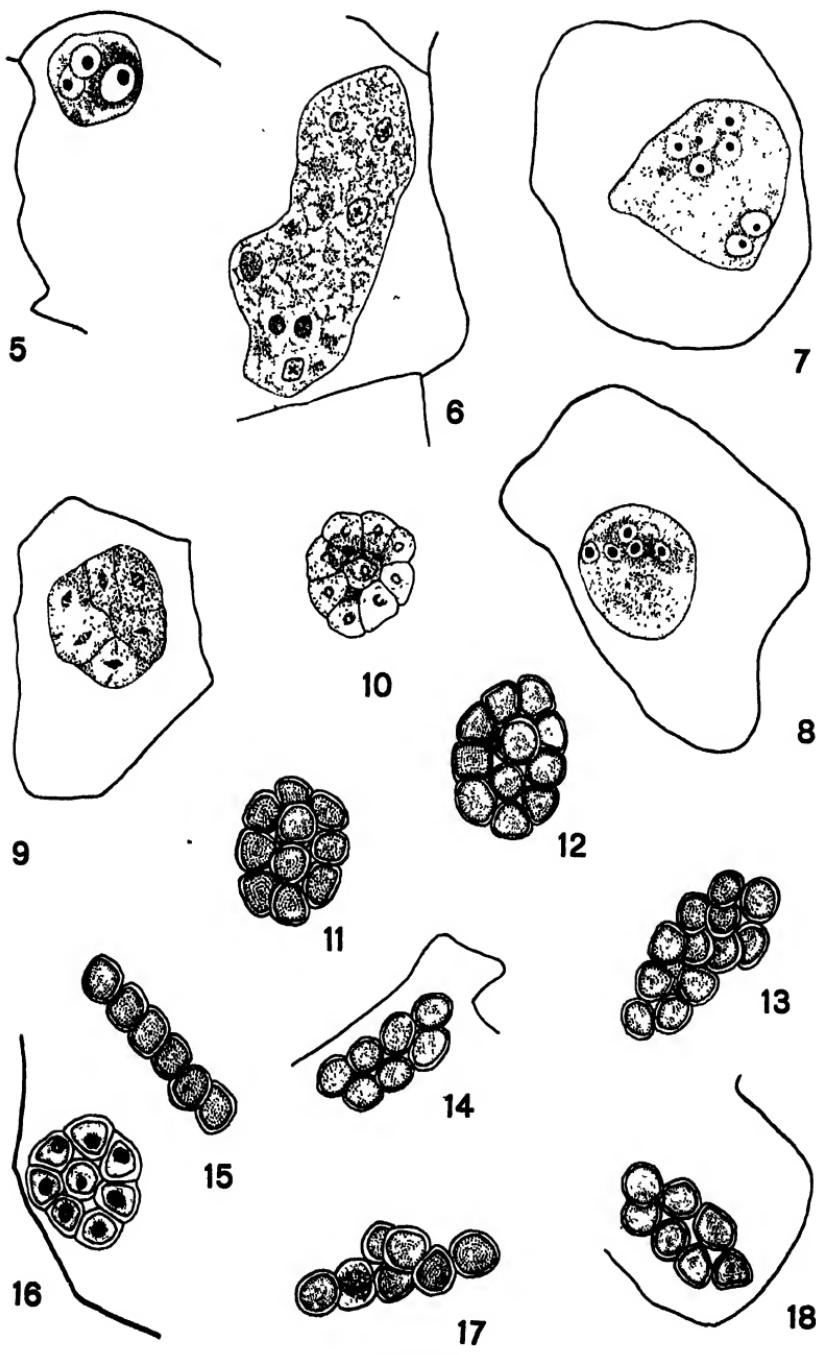
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#### EXPLANATION OF PLATES

##### PLATE 17

Photographs by W. R. Fisher. Fig. 1, galls on the true roots of *Heteranthera dubia*, nat. size; 2, galls on adventitious roots of the same host, nat. size; 3, a ribbon-like spore aggregation.  $\times 522$ ; 4, a long myxamoeba tending to encircle the host protoplast.  $\times 522$ .

##### PLATE 18

The drawings were made by the author and were outlined with the aid of a camera lucida. Apochromatic lenses were used.  $\times 1240$ . Fig. 5, a myxamoeba containing the large nuclei characteristic of the vegetative phase; 6, a myxamoeba showing the cruciform division figures characteristic of the growth period; 7, a myxamoeba in which the nuclei are about to undergo meiosis; 8, nuclei resulting from the first meiotic division; 9, second meiotic division accompanied by cleavage of the cytoplasm; 10, young thin-walled spores; 11, 12, disc-like spore aggregations, each composed of twelve spores; 13, spore aggregation lacking definite arrangement; 14, transverse section of a two-layered spore aggregation; 15, transverse section of a one-layered spore aggregation; 16, a single-layered eight-spored aggregation; 17, transverse section of a common type of spore aggregation in which the second layer of spores is incomplete; 18, transverse section of a two-layered sorus with open spaces between spores.

## STUDIES IN THE LEPTOMITACEAE. II. CYTOLOGY OF APODACHLYA BRACHYNEMA AND SAPROMYCES REINSCHII<sup>1,2</sup>

ARTHUR G. KEVORKIAN

(WITH PLATES 19 AND 20)

### INTRODUCTION

In the family Leptomitaceae cytological investigation had, until recently, been restricted to King's (1903) work on *Araiospora pulchra* Thaxter. However, in 1922 Guilliermond reported on the cytology of *Leptomitus lacteus* (Roth) Ag., and later Behrens (1931) on *Rhipidium europaeum* (Cornu) v. Minden. The writer has undertaken the study of representative species of the two remaining genera since a complete cytological survey of the group is desirable if the taxonomic interrelationship of the genera included in this family is to be worked out. The purpose of this paper, therefore, is to present the writer's cytological investigations of the genera *Apodachlya* and *Sapromyces*, thus making available additional data for a comparative study of the family as a whole.

### MATERIALS AND METHODS

The organisms in question were collected on twigs and other vegetable substrata in ponds and ditches at various points about Boston, Massachusetts, and Kingston, Rhode Island, during the spring and fall months of the year. In order to ensure sufficient material for a cytological study of the various stages of the life cycles, the fungi were grown on corn and barberries in cooled water cultures. Changes of water were made from time to time to offset the accumulations of bacteria.

For fixation, it was found after some experimentation that Merkel's solution, as modified by Smith (1923), gave the most

<sup>1</sup> The structure and development of a new aquatic Phycomycete. *Mycologia* 26: 145-152, 11 fig. 1934, is number 1 of this series.

<sup>2</sup> Contribution from the Cryptogamic Laboratories of Harvard University No. 135.

satisfactory results for the hyphae and nonsexual organs. In the case of the sex organs, a weak chrom-acetic acid killing solution, consisting of 7 cc. glacial acetic acid, 7 grams chromic acid, and 1000 cc. distilled water, gave the best results, especially when fixation was carried on from 12 to 15 hours. These two solutions were therefore used most extensively, although various other dilutions of chrom-acetic, with or without osmic acid, were used, as well as Bouin's fluid.

Material killed at regular intervals throughout the day showed that the time of day at which the fungus was killed was of importance since nuclear figures were found to be more abundant in all parts of the organism during the period between 10 p.m. and 3 a.m. than at other times during the day and night.

Several staining methods were tried, including Gram's method as suggested by Couch (1932), Delafield's haematoxylin, according to Chamberlain (1930), and Heidenhain's iron-alum-haematoxylin, with or without counterstains such as eosin and light green. Each of these stains was useful for the study of certain structures, since Gram's stain gave nuclear details, whereas haematoxylin stained other cytoplasmic details as well as the nucleus. Staining, dehydration in a graded alcohol series, clearing in xylol, and embedding in paraffin were accomplished in the usual manner, except that shorter intervals (30 min.) were used in dehydration, since it was found this could be done without causing plasmolysis or any other perceptible ill effects in the material in question.

Serial sections of material embedded in paraffin were cut 5, 10, or 12  $\mu$  thick of all the developmental stages of the organism; furthermore, portions of the thalli and asexual organs were mounted "in toto," as such mounts showed some gross features especially well, and moreover were helpful in interpreting the structures found in the sections.

In the case of exceptionally heavy walled structures into which paraffin penetrates with difficulty, structures such as the mature oospores of *Sapromyces*, the best results were obtained by embedding in celloidin in the manner outlined by Jeffrey (1928) and Wetmore (1932). Although celloidin<sup>1</sup> has not hitherto

<sup>1</sup> The writer is greatly indebted to Dr. H. W. Jensen and Mr. W. A. Crooks for helpful assistance in the use of the celloidin technique.

to been used to any extent for the study of aquatic fungi, the writer felt it merited a trial because of its successful use in embedding heavy walled cells of higher plants which present difficulties similar to those encountered in the mature oöspores of *Sapromyces*.

#### OBSERVATIONS

##### APODACHLYA BRACHYNEMA (HILD.) PRINGSHEIM

Of the genus *Apodachlya*, the two species *A. pyrifera* and *A. brachynema* were collected and the latter studied in detail in order to cast further light on the question of whether the "Dauer-sporangien" should be interpreted as gemmae, as they were by Pringsheim (1883), or interpreted as oögonia, as has been done by other authors.

*Thallus.*: The numerous nuclei scattered throughout the periphery of the protoplasm consist of a dark staining, more or less central portion, or nucleolus, about which there is a hyaline nucleoplasm, surrounded by a nuclear wall. Usually there are a varying number of linin strands radiating from the nucleolus to the nuclear wall, where they become slightly thickened at the point of attachment.

The seven to twenty-five nuclei of each segment are more numerous and usually spherical at the growing tips. This is in agreement with Smith's (1923) findings in the mycelium of the genus *Saprolegnia*.

*Sporangia.* Since no evidence of nuclear division was observed in the sporangium, the writer is led to believe that the inflow of nuclei with protoplasm from the supporting segments accounts for the number contained in the young sporangium. The six to twenty-three nuclei, which are at first scattered throughout the entire sporangium, are carried to the periphery when this organ becomes vacuolate. The nuclei are usually spherical, slightly larger than those of the mycelium, and take a somewhat deeper stain.

When spore-differentiation takes place within the sporangium, each individual nucleus is surrounded by a portion of the protoplasmic mass and separated from its neighbors by a system of clefts. The resultant densely granular, somewhat angular masses finally swell and fill the entire sporangium, and, when

completely differentiated, the zoospores are discharged through the papilla of dehiscence.

*Zoospores.* At the time of discharge, the single nucleus of each somewhat elongate zoospore is centrally located and measures about  $3 \mu$  in diameter. However, when the zoospore becomes typically pear-shaped, the nucleus is usually near the slightly pointed apex (PLATE 19, FIG. 9A) but in the ensuing stage when the zoospore rounds up and comes to rest, the nucleus again becomes central. Later, when from the encysted primary spore the secondary biciliate, kidney shaped zoospore emerges, the nucleus within it remains in the same central position (PLATE 19, FIG. 9B). In both types of spores the cilia are associated with the nucleus by slender fibrillar blepharoplasts, which, as described for *Chlamydomonas* by Kater (1929), perhaps remain distinct but give the appearance of being fused in a single structure (PLATE 19, FIG. 9). The nuclei are usually spherical, though occasionally angular, a condition which Cotner (1930) observed in this species, and Mathews in *Leptolegnia* (1932). Upon germination the single nucleus of the zoospore divides repeatedly, giving rise to a multinucleate germ tube.

*Sexual Organs.* At the ends of short lateral branches (PLATE 19, FIG. 1) are borne spherical bodies which have been interpreted as resting bodies by some authors, while others have called them oögonia subtended by an antheridium. The cytological procedure in these structures has convinced the writer that the single terminal cell is an oögonium and the cell below is a hypogynous antheridium. On sectioning and staining this hypogynous cell, the three to four dark-staining, spherical nuclei which are scattered in the protoplasm are found to be quite similar to those of the sporangium in size and structure (PLATE 19, FIG. 1). They then undergo a simultaneous mitotic division (PLATE 19, FIG. 2) and one of the resulting nuclei enlarges, while the others degenerate and the cytoplasm becomes somewhat vacuolate. Meanwhile the membrane separating the oögonium from the antheridium displays a perforation, but as in *Brevilegnia diclina* (Cooper 1929) and *Leptolegnia caudata* (Couch 1932) a definite fertilization tube is lacking. The functional male nucleus, together with a portion of the protoplasmic con-

tents of the hypogynous cell, now enters the terminal oögonium (PLATE 19, FIG. 4).

Since the several nuclei of the hypogynous cell all undergo a mitotic division and one of the resulting nuclei enlarges at the expense of the others, and since this is discharged into the oösphere while the rest degenerate, these points seem to indicate that Coker (1923) is correct in assuming that this organ is an antheridium.

Into the spherical, developing oösphere, meanwhile, ten to twenty nuclei are carried with the inflowing protoplasm. At first the nuclei, which are from 2.5 to 3.5  $\mu$  in diameter, are scattered more or less evenly throughout the cytoplasm (PLATE 19, FIG. 1); later, when the oösphere reaches its maximum size and a delimiting membrane is laid down, the nuclei undergo mitosis (PLATE 19, FIG. 2). Although all the nuclei are in process of division simultaneously with those of the antheridium, they may not all be at the same stage of division (PLATE 19, FIG. 2, 3). All but one of the resulting nuclei are then carried to the periphery, possibly by the activity of the vacuoles. Patterson (1927), working with *Achlya colorata*, also found that all but one of the nuclei occupied the peripheral regions of the oögonium, and he consequently designated this phase as the peripheral stage. The nucleus which remains in the center of the oögonium then enlarges, becoming two to three times its original size, while those at the periphery degenerate (PLATE 19, FIG. 4). While this is the general rule, in some cases the nuclei have been seen to divide and degenerate without migrating to the periphery, with the exception of the one which remains in the center.

Surrounding the centrally located female gamete-nucleus there now may be seen in many cases a finely granular, not too pronounced, irregular area which is apparently the coenocentrum described by various writers in certain of the Saprolegniales and Peronosporales. Meanwhile the male gamete-nucleus is liberated by the antheridium, and travels toward the egg nucleus (PLATE 19, FIG. 4), and upon coming in contact, both enlarge (PLATE 19, FIG. 5) and become rather closely appressed. At this stage several dark-staining linear condensations of cytoplasm are seen radiating outward from the periphery of the nucleus

(PLATE 19, FIG. 6, 7). These structures have been called astral rays by various workers and are found in closely related groups such as the Saprolegniaceae and the Peronosporaceae. The spherical oosphere now contains the enlarged functional male and female nuclei and the degenerating peripheral nuclei (PLATE 19, FIG. 6) which soon disappear completely.

Fusion is evidently a slow process, since the oospore wall may become clearly defined and increase in thickness before the male and female nuclei lose their identity (PLATE 19, FIG. 7). A similar delayed fusion was also noted by Stevens (1901, 1902), Trow (1901), and Patterson (1927) in representatives of related groups.

That the so-called resting sporangia or "Dauersporangien" are in reality oogonia and the sub-terminal cells antheridia seems clear, since the procedure mentioned above, namely, the division of the nuclei in the young antheridium and the young oogonium, the degeneration of the supernumerary nuclei in both organs, the subsequent migration of a single male gamete-nucleus into the oosphere, and eventually its fusion with the centrally located female gamete-nucleus, seems conclusive evidence that these bodies are sexual organs.

The fate of the fusion nucleus could not be followed during the early stages of oospore germination since the characteristic large oil globule which usually occupies the central portion of the mature oospore made observation difficult. Although this point has not been clarified, it may be assumed, however, that, as in the Monoblepharidales (Laibach 1927), reduction division is one of the several divisions which occur upon germination, giving rise to multinucleate germ tubes. This is accomplished after a resting period of several weeks.

#### SAPROMYCES REINSCHII (SCHRÖTER) FRITSCH

Although material of the two known species of the genus *Sapromyces* was collected and subjected to a comparative study, it seemed more fitting to present a cytological account of *S. Reinschii* since previously it has been the less studied of the two.

*Thallus.* In the basal cell, which develops from the germinating zoospore, the nuclei are scattered throughout the periph-

eral portion and are so numerous that an accurate count can be made only with difficulty. This multinucleate condition results from the repeated divisions of the single zoospore nucleus during the period of germination. In shape, the nuclei are slightly elongate ( $3.5$  to  $4 \times 5 \mu$ ) or spherical ( $4 \mu$  in diameter) and structurally similar to those already described for *Apodachlya brachynema*. In older plants they are not so numerous and are more elongate, especially those which are found in the basal cells.

*Sporangia.* The large spherical nuclei are carried into the sporangia by the inflowing protoplasm from the adjacent segments, a procedure similar to that described in *Apodachlya brachynema*.

In young sporangia the large spherical nuclei are scattered throughout the entire content, but after the small vacuoles have coalesced to form a large central vacuole, the nuclei assume a peripheral position. Upon further differentiation, the 12 to 30 nuclei of the sporangium are separated into individual spore initials by a number of slender clefts of vacuolar origin. Further details of the process of spore formation are similar to those described for *Apodachlya*.

*Zoospores.* The centrally located, spherical to slightly angular, single nucleus of the zoospore (PLATE 20, FIG. 2A), like the nuclei of the thallus, is enclosed by a finely granular, rarely vacuolate central cytoplasmic area which is in turn surrounded by the coarsely granular, rather vacuolate, peripheral cytoplasm containing irregular densely-staining granules, and the whole surrounded by a thin limiting membrane.

The two lash-like cilia, which are about four times as long as the spore body, one directed forward, and the other backward, have their origin in the lateral groove of the zoospore and are connected to the nucleus by an arrangement of basal granules, similar to those previously described in *Apodachlya* and also for *Rhipidium* (Cotner 1930) and *Araiospora* (Kevorkian 1934).

*Sexual Organs.* The curved, oblong antheridium twines about the oögonium and attaches itself at its apex by means of a beak-like process. In this body the four to six nuclei are similar in structure to those of the sporangia and oögonia, but after a short time they undergo one mitotic division (PLATE 20, FIG. 4) and

one of the resulting nuclei, usually more deeply-staining, enlarges, while the rest gradually degenerate. In the meantime the antheridium pushes in a beak-like process at the point of attachment. This definite, rather thick-walled fertilization tube enters the oosphere and discharges its male gamete-nucleus, allowing it to migrate toward the centrally located egg nucleus (PLATE 20, FIG. 5).

In the young oögonial initial the ten to twelve spherical nuclei which are carried in with the inflowing protoplasm are scattered throughout the protoplasm (PLATE 20, FIG. 3, 6), as in *Apodachlya*; the oögonium soon becomes vacuolate and the nuclei undergo one mitotic division, somewhat similar to that which occurs in the genus *Araiospora* (King 1903), and all but one migrate to the periphery (PLATE 20, FIG. 4, 6). In certain other instances, however, all but the selected female gamete nucleus degenerate without going to the periphery (PLATE 20, FIG. 11, 12). Behrens (1931), referring to *Rhipidium*, states: "Wenn das Oogon seine volle Grösse erreicht . . . haben sich die Kerne in ziemlich regelmässigen Abständen über die ganze Peripherie verteilt, mit Ausnahme eines einzigen, der in der Mitte der Oogons verbleibt." This observation seems to agree with what the writer finds in *Sapromyces*. The remaining, more or less centrally located nucleus enlarges to from 6 to 8  $\mu$  in diameter at the expense of the others which, as stated above, degenerate (PLATE 20, FIG. 5-7), a process which also takes place in certain of the Peronosporaceae (Berlese 1897). The separated periplasm, which usually contains the peripheral nuclei, gradually changes in texture and soon gives rise to a membrane separating the more granular oöplasm from the less granular periplasm (PLATE 20, FIG. 9). At this stage of development, as in *Apodachlya*, the so-called "coenocentrum" makes its appearance in the central portion of the oögonium (PLATE 20, FIG. 8). This finely granular and less vacuolate area soon disappears and the enlarged, rather closely associated male and female nuclei can be seen more distinctly (PLATE 20, FIG. 10, 11). After some delay these fuse, forming a much enlarged, single, centrally located nucleus in the mature oöspore (PLATE 20, FIG. 12). As is the case in numerous other watermolds, the further fate of the fusion nucleus is not known, since germination has never been observed in *Sapromyces*.

## LEPTOMITUS LACTEUS (ROTH) AGARDH

The cytology of *Leptomitus lacteus* was worked out in the same detail as that of *Apodachlya* and *Sapromyces*, but since sexual organs have not been reported in the genus, the observations were necessarily confined to the mycelium and non-sexual organs. The oval to elongate nuclei, which are usually located in the peripheral protoplasm of the mycelium, are from 2 to 3  $\mu$  in diameter and somewhat similar to those of *Apodachlya* and *Sapromyces*. The nuclei within the sporangia average about twenty to thirty in number, are usually spherical, and possess a dark-staining nuclear membrane enclosing a dark, centrally located nucleolus surrounded by a hyaline zone. These findings only corroborate what has already been worked out by Büsgen (1882) and Dangeard (1890) and, being limited to the mycelium and sporangia, naturally furnish no data for comparison with the cytological details of the sexual organs or fertilization in the other genera.

## CONCLUSIONS

The cytological procedure in the so-called "Dauersporangien" of *Apodachlya brachynema* reveals the fact that these structures are in reality terminal oögonia and the sub-terminal cells antheridia. Although a number of authors such as Coker (1923), Sparrow (1932), Cejp (1932), etc., have suggested that these bodies, in structure, position, and behavior, gave indication of being sexual organs, cytological observations to support their morphological evidence of the true identity of these terminal organs has been lacking hitherto.

Unlike the other members of the family, the sexual organs of *Apodachlya brachynema* are more closely allied to such forms as *Leptolegnia* and *Brevilegnia* of the Saprolegniaceae than those of the Leptomitaceae, in that there is no definite separation of the protoplasmic content of the oögonium into an inner oöplasm and outer periplasm. Furthermore, the oöspore of *Apodachlya*, at maturity, lacks a definite exospore such as exists in *Sapromyces*, *Araiopora*, and *Rhipidium*. In *Sapromyces*, on the other hand, the cytological procedure is very similar to that of certain members of the Peronosporales, notably *Sclerospora*, *Pythium*, *Peronospora*, etc., as studied by Stevens (1902), Trow (1901), and Ber-

lese (1897), in that the oögonium containing the scattered nuclei becomes vacuolate and a nuclear division takes place. Later all but one of the resultant nuclei migrate to the less granular periplasm and there degenerate. The selected male nucleus discharged by the fertilization tube then fuses with the enlarged female nucleus located in the densely granular öoplasm.

These data, together with certain morphological observations (Kevorkian, unpublished) such as the Saprolegnoid habit, diplanetic zoospores and oöspore devoid of a definite exospore, lead the writer to believe that *Apodachlya* and its ally *Leptomitus* are transitional forms between certain members of the Saprolegniaceae and Leptomitaceae, whereas *Sapromyces* and its allies are more closely related to the Peronosporales than the Saprolegniales.

In conclusion the writer wishes to acknowledge his indebtedness to Professor W. H. Weston, Jr., under whose supervision this research was done, for his constant interest, helpful assistance, and stimulating criticism; to Dr. D. H. Linder for many valuable suggestions throughout this work; and to Dr. R. M. Whelden for helpful assistance pertaining to cytological procedure.

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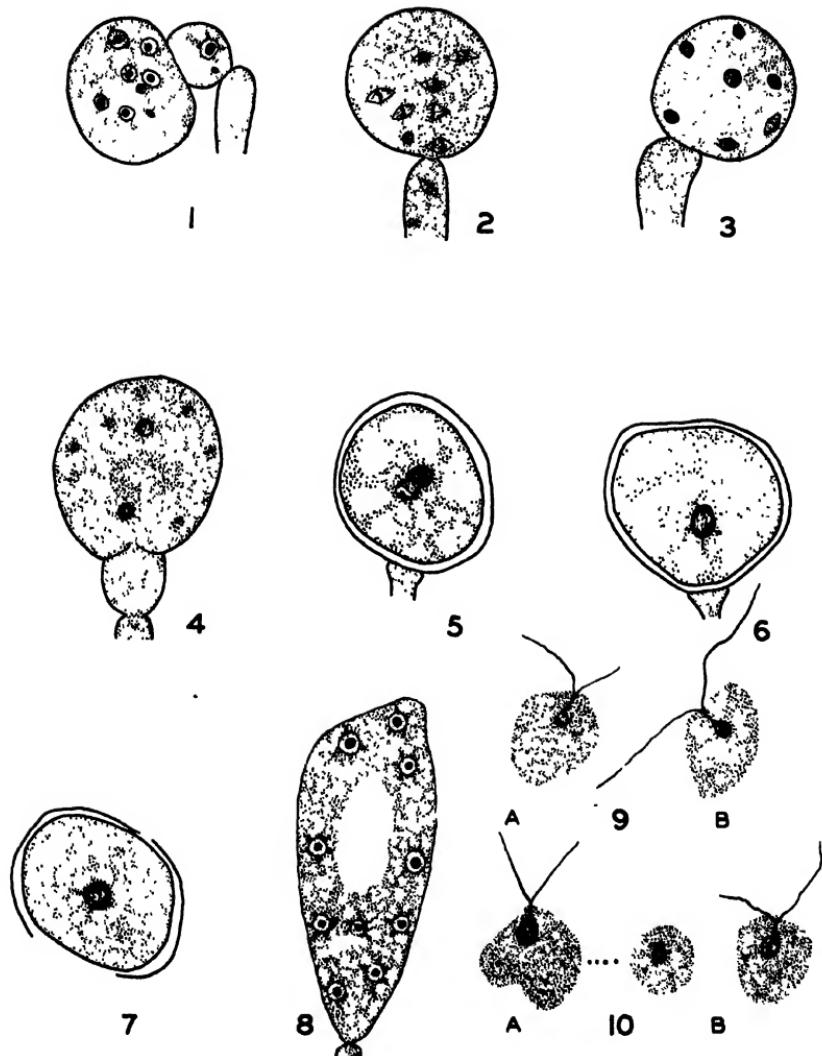
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#### EXPLANATION OF PLATES

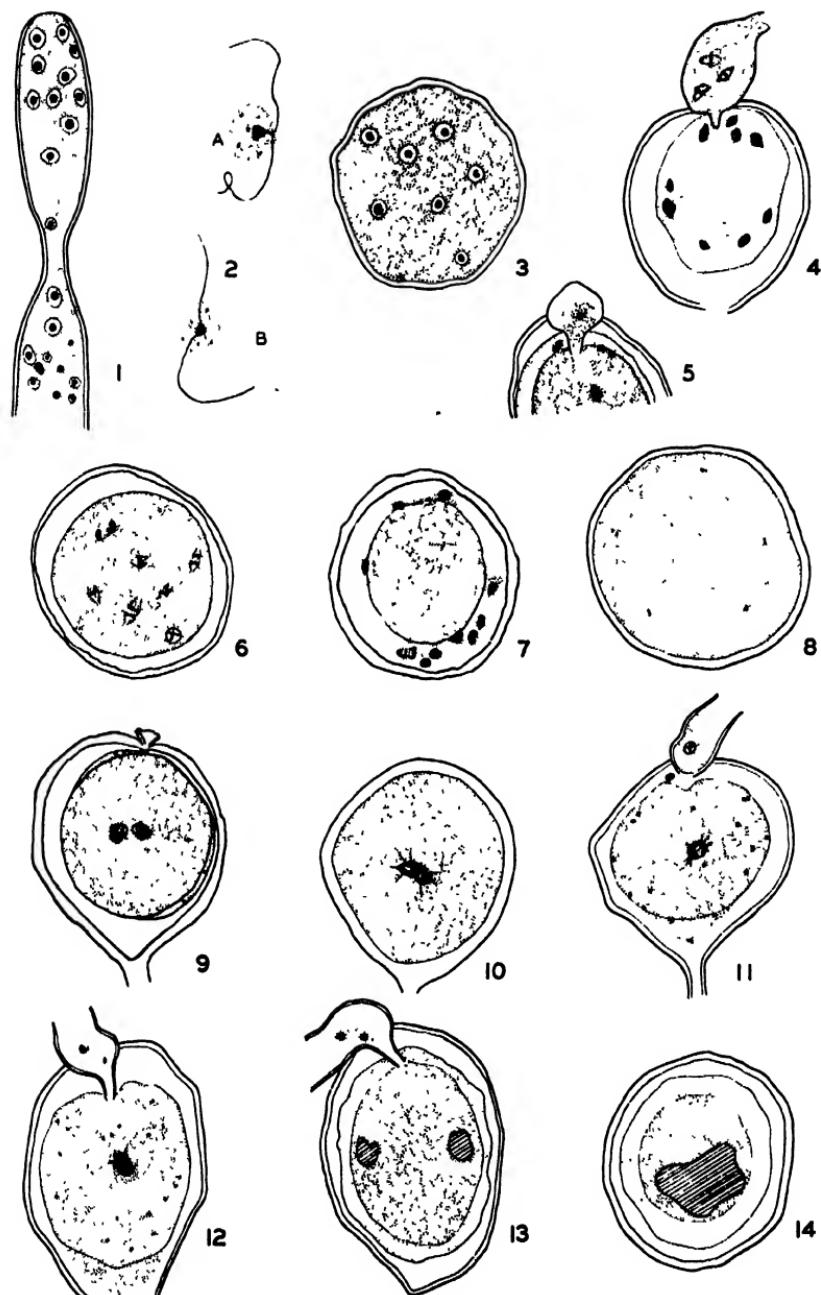
The figures were drawn from killed and fixed material with the aid of an Abbe Camera Lucida with a 10 X ocular and 1.25 mm. objective. Approximate magnification X 800 for Plate 19 and X 500 for Plate 20.

#### PLATE 19

*Apodachlya brachynema*: Fig. 1, young oögonium and antheridium showing arrangement of nuclei; 2, mitosis in the oögonium and antheridium; 3, oögonium showing peripheral arrangement of nuclei before degeneration; 4, oögonium showing male and female nuclei and degenerating nuclei at periphery. Coenocentrum is present; 5, oögonium with thickened oögonial wall, showing association of centrally located male and female nuclei; 6, oögonium showing nuclei with astral rays prior to complete fusion; 7, fusion nucleus with astral

1-9. *APODACHYLA BRACHYNEMA*10. *LEPTOMITUS LACTEUS*





SAPROMYCES REINSCHII



rays; 8, sporangium showing arrangement of nuclei; 9, A. zoospores of the primary type; B. zoospores of the secondary type; 10, *Leptomitus lacteus*: A. zoospores of the primary type; B. zoospores of the secondary type.

#### PLATE 20

*Sapromyces Reinschii*: Fig. 1, nuclear distribution in very young sporangium; 2, A and B. zoospores; 3, nuclear distribution in early stage of oögonium; 4, peripheral arrangement of oögonial nuclei prior to division, antheridial nuclei at metaphase; 5, migration of the chosen male nucleus; 6, oögonial nuclei dividing before migration to periphery; 7, degeneration of peripheral nuclei; 8, oögonia showing coenocentrum; 9 and 10, male and female nuclei prior to fusion; 11, male and female nuclei in close association showing astral rays, supernumerary nuclei in process of degeneration; 12, oögonium showing fusion nucleus; 13 and 14, mature oöspores showing oil globules.

# CONIDIAL FORMATION, MUTATION AND HYBRIDIZATION IN PENIOPHORA ALLESCHERI<sup>1</sup>

MILDRED K. NOBLES

(WITH PLATES 21-23 AND 4 TEXT FIGURES)

Preliminary examination of cultures of *Peniophora Allescheri* Bres. showed that the fungus produces abundant conidia on oedcephaloid heads on both haploid and diploid mycelia. Observations of the occurrence of similar structures in the Hymenomycetes have been reported by Brefeld for *Tomentella flava* Brefeld and *T. granulata* Brefeld (1), for *Fomes annosus* (Fries) Cooke by Brefeld (1), Lyman (6) and others, and for *Corticium roseo-pallens* Burt and *C. effuscatum* Cooke and Ellis by Lyman (6), but these studies were made prior to the establishment of modern conceptions of interfertility phenomena in Basidiomycetes. Hence, in most cases, it is impossible to determine from the data whether the conidia were produced on haploid or diploid mycelia, or the value of the mycelium obtained by their germination.

Other types of conidial or oidial formation have frequently been reported among the higher Basidiomycetes, and in a few cases some analyses of the spores have been made. Accessory spores produced on the haploid mycelium repeat that generation, and in some instances spores produced on the diploid mycelium repeat that phase, as is the case in *Polyporus squamosus* Huds. and *Pleurotus pinsitus* Fries (10). This is not universal. Gilmore (2), working on *Psilocybe coprophila* Fries, showed that the diploid mycelium produced oidia, some of which gave rise to haploid, some to diploid mycelia. Martens and Vandendries (7), studying *Pholiota aurivella* Batsch., found that the conidia developed on specialized branches on the diploid mycelium were either diploid or haploid in value, the latter arising by the division and disjunction of a binucleate spore in such a way as to give rise to two uninucleate spores.

<sup>1</sup> Contribution from the Department of Botany, University of Toronto.

Since no detailed study had been made of conidial formation in a Basidiomycete having the Oedocephalum type of conidiophore, such a study was undertaken of *Peniophora Allescheri* to determine, first, stages in conidial formation and second, the value of the conidia.

	A										B		
	2	7	9	10	23	24	25	27	28	16	19	22	
A	—	—	—	—	—	—	—	—	—	+	+	+	
	—	—	—	—	—	—	—	—	—	+	+	+	
	—	—	—	—	—	—	—	—	—	+	+	+	
	—	—	—	—	—	—	—	—	—	+	+	+	
	—	—	—	—	—	—	—	—	—	+	+	+	
	—	—	—	—	—	—	—	—	—	+	+	+	
	—	—	—	—	—	—	—	—	—	+	+	+	
	—	—	—	—	—	—	—	—	—	+	+	+	
	—	—	—	—	—	—	—	—	—	+	+	+	
	—	—	—	—	—	—	—	—	—	—	—	—	
B	+	+	+	+	+	+	+	+	+	—	—	—	
	+	+	+	+	+	+	+	+	+	—	—	—	
	+	+	+	+	+	+	+	+	+	—	—	—	

TABLE I. Results obtained by pairing in all possible combinations twelve single basidiospore cultures of *Peniophora Allescheri*. A plus (+) sign indicates the presence of clamp connections, a minus (−) sign their absence.

The fruiting body from which the cultures were originally obtained was collected on bark of *Populus* sp. by Professor H. S. Jackson, near Aurora, Ontario, on October 22, 1933 (U. of T. Herb. No. 5605). It was identified as *Peniophora Allescheri* Bres. by Dr. L. O. Overholts.

## CULTURES FROM BASIDIOSPORES

The fruiting body yielded basidiospores which, when sown on plates of cooled malt extract agar, germinated in from two to three days. Isolated colonies were transferred to stock culture tubes containing malt extract agar and in this way twenty single basidiospore cultures were obtained.

When grown on 2 per cent malt extract agar at room temperature, the colony resulting from a single basidiospore is slow growing (FIG. 1*b*) with most of the mycelium submerged or appressed, and with scanty aerial growth. Microscopic examination shows that the hyphae have simple septa, without clamp connections. This condition has persisted through several transfers over the six months the fungus has been in culture.

While the colonies are still microscopic in size, conidial production begins. The conidia are produced on specialized conidiophores which may be either apical or lateral with respect to the hyphae. The typical conidiophore (FIG. 3, 4, 5) is a rounded or club-shaped structure 9–14  $\mu$  in diameter, at first smooth, later developing slender tapering sterigmata, up to 2  $\mu$  in length, on which the conidia are borne. The number of conidia on a head varies from eight to thirty-two, although occasional conidiophores have only four conidia. The conidiophore, which is separated from the hypha by a septum, becomes vacuolate as the spores develop, and finally empty, the contents all passing into the spores. In plate cultures the spores remain in a mass on the collapsed conidiophore; in hanging drop cultures they float away (FIG. 5).

The conidia vary in length from 12–15  $\mu$ , are hyaline, cylindrical and often somewhat curved.

Twelve haploid mycelia, each derived from the germination of a single basidiospore, were grown in pairs in all possible combinations, and the resulting mycelia examined microscopically for the presence of clamp connections. The results are incorporated in Table I,<sup>1</sup> in which a plus sign indicates the occurrence of clamp connections, a minus sign their absence. It will be observed that the mycelia fall into two groups, a member of

<sup>1</sup> In this paper the symbols A and B have been used to designate the two groups of mycelia, in place of the more conventional A and a.

group A reacting with a member of group B in such a way as to produce a mycelium bearing clamp connections. A member of one group is unable to react in this way with another member of the same group. *Peniophora Allescheri* is therefore genetically heterothallic, defining this term to mean that the thalli which react together to produce a clamp-bearing mycelium contain nuclei of different genetic constitution as regards the factors which determine for or against this reaction.

The diploid mycelium obtained by growing together two suitable mycelia, resembles the parent haplonts in rate of growth and character of colony (FIG. 2a) and in microscopic characters, except that the hyphae of the diploid mycelium have clamp connections at every septum. This mycelium, like the haploid, bears abundant conidiophores (FIG. 6) which are similar in appearance to those borne on the haplonts. The conidia developed on these conidiophores are not distinguishable from those produced on the haploid mycelium.

#### CYTOTOLOGICAL STUDY OF CONIDIAL FORMATION

A cytological study has been made of basidiospores, sporelings and haploid and diploid mycelium. For this purpose the material to be examined was used to inoculate a drop of 2 per cent malt extract solution on a coverslip and inverted over a van Tieghem cell. At the desired stage of development, the material was killed by adding a drop of formalin-acetic-alcohol or Bouin's fixative, and dried down on the coverslip. It was then stained in Haiden-hain's haematoxylin, dehydrated and mounted in the usual way.

The basidiospore is uninucleate and germinates to produce a germ tube which becomes septate as soon as the first nuclear division occurs. Thus the sporeling and haploid mycelium are strictly uninucleate from the beginning.

The first indication that a conidiophore is forming in a terminal position is a swelling at the end of the hypha. When the conidiophore occupies a lateral position it usually attains almost its final size before the nucleus of the cell from which it arises migrates into it. In the early uninucleate stage (FIG. 9) the contents of the conidiophore are homogeneous and densely staining.

By successive divisions two, four, eight, sixteen or more nuclei are formed within the head, the nuclei following each division appearing successively smaller than those resulting from the preceding division (FIG. 10, 11, 12). The nuclei take up their

	A							B				
	1	2	5	6	7	8	9	10	11	3	4	12
A	—	—	—	—	—	—	—	—	—	+	+	+
	—	—	—	—	—	—	—	—	—	+	+	+
	—	—	—	—	—	—	—	—	—	+	+	+
	—	—	—	—	—	—	—	—	—	+	+	+
	—	—	—	—	—	—	—	—	—	+	+	+
	—	—	—	—	—	—	—	—	—	+	+	+
	—	—	—	—	—	—	—	—	—	+	+	+
	—	—	—	—	—	—	—	—	—	+	+	+
	—	—	—	—	—	—	—	—	—	+	+	+
	—	—	—	—	—	—	—	—	—	+	+	+
B	+	+	+	+	+	+	+	+	+	—	—	—
	+	+	+	+	+	+	+	+	+	—	—	—
	+	+	+	+	+	+	+	+	+	—	—	—

TABLE II. Results obtained by pairing in all possible combinations twelve cultures from single conidia developed on diploid mycelium.

positions around the inner surface of the distal half of the conidiophore and the basal portion becomes vacuolate. At this stage the slender tapering sterigmata push out and a swelling, the developing conidium, appears on the end of each. While the spore is still small a nucleus migrates into it, becoming thin and elongated in order to pass through the narrow sterigma (FIG. 14). The conidium increases in size, the contents stain densely and appear homogeneous, and the nucleus increases in size (FIG. 13). Apparently all of the cytoplasm of the conidiophore is used up in

spore formation, for when all the conidia are mature, the head appears empty and finally collapses (FIG. 5).

The cells of the clamp-bearing mycelium are regularly binucleate, the nuclei undergoing conjugate divisions through the agency of the clamp connection. Conidiophore development proceeds in a manner similar to that in the haploid conidiophore. The conidiophore may occupy a terminal position, in which case it appears first as a swollen binucleate cell, or a lateral position, when it pushes out from the side wall of a cell and reaches a considerable size before the two nuclei of the cell migrate into it (FIG. 15). In either case, the conidiophore is at first binucleate (FIG. 15, 16). These two nuclei divide simultaneously but without the formation of a clamp connection (FIG. 17). Later stages show the presence of four, eight, sixteen or more nuclei in each head (FIG. 18). The formation of sterigmata and spores takes place as in the haploid conidiophores, and one nucleus migrates into each conidium. Thus the binucleate, clamp-bearing diploid mycelium produces uninucleate conidia (FIG. 19). The evidence from cytological study indicates that both the original nuclei have taken part in providing the daughter nuclei that finally migrate into the spores.

#### CULTURES OF CONIDIA FROM DIPLONT

As previously stated, the conidia produced on the binucleate mycelium are themselves uninucleate. This observation is substantiated by the results of culture work.

A pairing of single basidiospore cultures 1 and 12 was made by inoculating a plate of cooled malt extract agar with mycelium from each of the stock cultures. They grew together, the resulting mycelium bearing clamp connections. A transfer of hyphal tips from this clamp-bearing mycelium was made to an agar slant in a stock culture tube. Later a transfer from this diploid stock culture ( $1 \times 12$ ) was made to a plate of cooled malt extract agar. By this method, the possibility of haploid mycelium being carried over was reduced to a minimum.

Conidia are produced very abundantly on such a diploid colony. These conidia were used to inoculate hanging drop cultures of 2 per cent malt extract solution, in order to watch

germination. They germinated in from twenty-four to forty-eight hours at room temperature, to give sporelings with simple septa, indistinguishable from the sporelings derived from basidiospores. This indicated that the conidia produced on the

	A	B
	I	12
A	2	+
	7	+
	9	+
	10	+
	23	+
	24	+
	25	+
	27	+
	28	+
	16	-
B	19	-
	22	-

TABLE III. Results obtained by mating twelve single conidial cultures from a diploid mycelium with the two parent basidiospore cultures.

diploid clamp-bearing mycelium germinate to give haploid mycelia.

That this is the case was proved by further work. Conidia from this diploid colony ( $1 \times 12$ ) were sown on plates of cooled malt extract agar, and isolated colonies were transferred to agar slants in stock culture tubes. In this way twenty-five single conidial cultures were obtained. These cultures (with one exception to be discussed later) are identical with the cultures arising from single basidiospores, in gross appearance and absence of clamp connections.

Twelve of these single conidial cultures were grown in pairs in all possible combinations, and the resulting mycelia examined for the presence of clamp connections. The results are given in Table II where as before the plus sign indicates the presence of clamp connections, the minus sign indicates their absence. It will be observed that, as in Table I, the mycelia fall into two groups, a member of one group reacting with a member of the other group in such a way as to produce a mycelium bearing clamp connections.

Each of the twelve single conidial cultures was then paired with basidiospore culture 1 and basidiospore culture 12 and the resulting mycelia examined for the presence of clamp connections. The results are shown in Table III from which it is evident that conidial cultures 16, 19 and 22 are identical with basidiospore culture 12, and so belong to group B, while conidial cultures 2, 7, 9, 10, 23, 24, 25, 27 and 28 are identical with basidiospore culture 1 and so belong to group A.

While the cytological study indicated that both nuclei of the young conidiophore divide to provide the nuclei that migrate into the spores it seemed desirable to test this by determining whether the conidia from a single head are of the two kinds. Considerable difficulty was encountered in removing the spores from a single conidiophore, since the conidiophores were very closely crowded on the colony, and the spores were repelled by, or at least did not cling to, a fine steel needle which was used in the first attempts. Later it was found that the conidia could be successfully transferred by using a fine glass needle rubbed with silk in order to induce an electric charge. The spores were apparently attracted to this, and several conidia from one head could be removed to the surface of poured malt extract agar plates. Spores from eight single conidiophores borne on diploid clamp-bearing mycelium, have been allowed to germinate in groups, and in every case clamp-bearing mycelium has developed. This result clearly indicates that the conidia from a single conidiophore were of two kinds, and gave rise to two kinds of mycelia which were able to react in such a way as to produce diploid clamp-bearing mycelium.

As a result of cytological and cultural work, it may be con-

cluded that the diploid clamp-bearing mycelium produces conidia which are haploid in nature, that these conidia germinate to give haploid mycelia, and that these haploid mycelia react with one another and with the mycelia derived from single

	A						B					
	18	19	20	21	22	23	1	2	3	4	5	6
A	18	—	—	—	—	—	+	+	+	+	+	+
	19	—	—	—	—	—	+	+	+	+	+	+
	20	—	—	—	—	—	+	+	+	+	+	+
	21	—	—	—	—	—	+	+	+	+	+	+
	22	—	—	—	—	—	+	+	+	+	+	+
	23	—	—	—	—	—	+	+	+	+	+	+
B	1	+	+	+	+	+	—	—	—	—	—	—
	2	+	+	+	+	+	—	—	—	—	—	—
	3	+	+	+	+	+	—	—	—	—	—	—
	4	+	+	+	+	+	—	—	—	—	—	—
	5	+	+	+	+	+	—	—	—	—	—	—
	6	+	+	+	+	+	—	—	—	—	—	—

TABLE IV. Results obtained by pairing in all possible combinations twelve cultures from single conidia developed on normal X non-conidial hybrid.

basidiospores in precisely the same way as the single basidiosporal mycelia react. Furthermore both A and B conidia are produced on one conidiophore.

#### MUTATION AND HYBRIDIZATION

As mentioned earlier, one single conidial culture differed from all others in macroscopic and microscopic characters. This culture was obtained from the germination of a single conidium which had been produced on a normal diploid clamp-bearing

mycelium resulting from the mating of two normal haploid mycelia (basidiospores 1 and 12). Of the twenty-five single conidial cultures isolated, twenty-four resembled the parents in type of colony formed and in microscopic characters, while one culture was noticeably different. This culture grew much more rapidly than either of the parent cultures (FIG. 1a) and produced more abundant aerial mycelium, so that the colony appeared cottony and much whiter than the normal colony (compare FIG. 1a and 1b). Microscopic examination revealed an entire absence of conidiophores and the presence of many short curved branches which are probably responsible for the cottony appearance of the colony (FIG. 7). These characters have persisted through the three months since this culture was obtained, and have remained constant through several transfers of the mycelium.

It would seem, therefore, that this non-conidial form is to be interpreted as a mutant in the haploid, having originated as the result of some nuclear change which occurred during the formation of the conidium or in nuclear divisions immediately preceding conidial formation.

This non-conidial mutant (number 22 in Tables II and III) has the ability to form hybrids<sup>2</sup> when paired with suitable haploid mycelia. From Table II it is seen that it reacts in such a way as to form clamp-bearing mycelium with conidial cultures 2, 7, 9, 10, 23, 24, 25, 27 and 28. From Table III it is clear that the mutant 22 belongs to the same group (group B) as the parent basidiospore culture 12, and has the ability to form clamp-bearing mycelium when mated with basidiospore culture 1 (group A). Therefore the mutation has not affected the ability to react in such a way as to form clamp-bearing mycelium when mated with a haplont of opposite group.

The diploid clamp-bearing mycelium obtained by mating the

<sup>2</sup> The term "hybrid" is commonly used to describe the first diploid generation obtained by the pairing of gametes produced by two dissimilar diploid parents. In the present paper the term is used to refer to the thallus (dicaryophyte) obtained by mating two dissimilar haplotypes. The nuclei in the cells of such a dicaryophyte remain separate until the fusion in the basidium. While the situation is obviously different than in truly diploid organisms, it has seemed best for the purposes of this discussion to use the term "hybrid" in this general sense, rather than to introduce a special terminology.

non-conidial mutant with a suitable haplont originating either from a basidiospore or a conidium, differs from the normal diploid mycelium resulting from the pairing of two normal haploid mycelia. Macroscopically the hybrid cross resembles the mutant parent in that it grows at approximately the same rate and develops considerable aerial mycelium, so that the resulting colony has an appearance similar to that of the mutant (FIG. 2b). Microscopic examination shows that the clamp-bearing hyphae exhibit the same type of branching as that of the mutant (FIG. 8), this apparently being associated with the cottony appearance of the colony. Unlike the mutant, however, the diploid colony produces conidiophores and conidia, in this respect resembling the normal parent, although the formation of conidiophores takes place at a somewhat later stage in the development of the colony and in less abundance than on the normal.

Such a hybrid cross was obtained by growing together non-conidial mutant 22 (group B) and single conidial culture 24 (group A), and the same technique as was described above was used to insure the purity of the diploid clamp-bearing mycelium. Conidia from this culture were sown on plates of cooled malt extract agar. While the colonies were still small (up to 3 mm. in diameter) they were examined, using the low power of the microscope. The two types of colony were readily distinguishable, the normal type being small and producing conidia while still minute in size, the mutant type being relatively larger and without conidia. In this way two hundred and ninety-five colonies were examined. Of this number, 142 were of the normal conidia-producing type like the parent culture 24, 153 were of the non-conidial type resembling the mutant parent 22.

Numerous attempts were made to germinate separately the conidia from an individual conidiophore, but many difficulties have been encountered. However, in one case two conidia from a single head germinated, and the two mycelia produced were isolated. One of these resembles the normal parent in growth characters and conidial production, the other the non-conidial parent. When paired, these two mycelia give rise to a typical hybrid cross colony. This, along with the evidence obtained by examination of many colonies resulting from conidia borne on

the hybrid cross, shows that both the original nuclei, one bearing the factor for conidial production, one for conidial sterility, were involved in the formation of conidia on a single head.

Twenty-four of these single conidial cultures were isolated, twelve of the normal conidial type, twelve of the non-conidial type. These have remained constant.

Six of the non-conidial haploid mycelia were paired in all possible combinations with six of the normal haploid mycelia, the results being tabulated in Table IV. It is evident that the non-conidial mycelia 1 to 6 fall into one group, conidial mycelia 18 to 23 into another group. An individual of the first group when paired with an individual of the second group is able to react in such a way as to form clamp-bearing mycelium. Since the non-conidial mycelia are all of the same reaction type it may be assumed that they are identical with non-conidial parent 22, and similarly that the conidial mycelia are identical with normal parent 24.

#### DISCUSSION

It has been established that in *Peniophora Allescheri* unicellular conidia are produced on both haploid and diploid mycelia. The spores from the haploid serve to repeat that generation, those from the diploid re-establish haploid mycelia identical with the two types of haploid mycelia which entered into the formation of the diplont. Thus new haploid mycelia and new hybrids can be formed at all stages during vegetative activity. If mutations occur in nature as seems possible since a noticeable one has arisen during the short time this fungus has been under observation, then the mechanism exists for including such mutants into diploid hybrids immediately. Furthermore, the two types of haplonts entering into the diplont could be recovered through the haploid conidia produced on it. This would allow for repeated hybridizations and so make possible the establishment of many such hybrids in place of the original one.

It has been stated previously that the diploid clamp-bearing mycelium resulting from the pairing of the non-conidial mutant with a normal conidial haplont resembles the non-conidial parent in macroscopic appearance and excessive branching, and the

conidial form in production of conidia. Cytological work shows that such a clamp-bearing mycelium is binucleate and it is accepted that in each pair one nucleus is a descendant of a nucleus derived from one parent, the other a descendant of a nucleus derived from the other parent. It may be assumed that in such a cross the nucleus from the non-conidial parent is influencing the type of development of the diploid mycelium in such a way that it resembles that parent in rapidity of growth and excessive branching. On the other hand the nucleus from the normal conidia-bearing parent is influencing the type of development of the diploid mycelium so that it resembles the normal parent in the production of conidia. This, then, indicates clearly that the two nuclei of the pair, though existing independently in the cells, coöperate in determining the characters of the diploid colony. The effect would appear to be comparable to that normally expected in a first generation hybrid in plant and animal groups having diploid nuclei. The diploid thallus obtained by mating two different haplonts shows biparental characters.

Binucleate hybrids in rusts and smuts have been studied and there also, the two nuclei of the pair each share in determining the characters of the diplont. Using pathogenicity as a criterion Newton, Johnson and Brown (8) found that hybrids formed by crossing physiologic forms of *Puccinia graminis Tritici* were "intermediate in pathogenicity between the two parental forms" and Levine and Cotter (5), by crossing strains of *P. graminis Secalis* and *P. graminis Tritici*, obtained a strain of stem rust capable of attacking certain varieties of rye and wheat which neither of the parents alone could do. In comparable experiments in smuts Goldschmidt (3) hybridized two strains of *Ustilago violacea*, each of which was able to infect only one host, and produced a diplont capable of attacking both hosts. Using morphological characters as a criterion, Newton, Johnson and Brown (9) observed that in a cross of strains of *P. graminis Tritici* involving two spore color types, one being present in each parent, the resulting hybrid showed the presence of both characters.

In all these cases studies have been made on binucleate hybrids and it has been established that such hybrids combine the

characters of the parents. It may be concluded, therefore, that in crosses such as these and in *Peniophora Allescheri*, in which two nuclei are associated but not fused, both of the nuclei are effective in determining the nature of the resulting diplont.

Subsequent analyses of the progeny of such hybrids have been made by Zattler (11) on *Schizophyllum commune* Fries and *Collybia velutipes* Curt. and by Johnson, Newton and Brown (4) on *Puccinia graminis Tritici*. Up to the present time the basidiospores of *Peniophora Allescheri* have not been obtained in culture. Observation of the hymenium formed in nature shows characteristic fusion of nuclei in the basidia and subsequent production of four nuclei and four uninucleate spores. If formation of perfect fruiting bodies in the hybrid can be obtained in culture, analyses of the progeny will be undertaken.

It should be emphasized, in conclusion, that the study of the hybrid in *Peniophora Allescheri* has been confined to an analysis and comparison of the vegetative characters and conidial production of the two haplonts involved in the cross, with similar characters of the resulting hybrid dicaryophyte or diplont. In the papers cited above the comparison of characters is between two generations of the diplont, the characters of the intervening haplonts not having been considered. A heterothallic basidiomycete, such as *Peniophora Allescheri*, in the vegetative phase, may be considered as having an alternation of "homologous" haploid and diploid generations. Since these may be grown separately in pure culture it is possible to compare vegetative characters of the haploid thalli with corresponding characters of the diplont. Such a direct comparison would not appear to be possible in other groups of the fungi, though certain green, brown and red algae having an alternation of homologous generations would seem to offer similar opportunities.

#### ACKNOWLEDGMENT

This study has been carried on under the direction of Professor H. S. Jackson, to whom I wish to express my appreciation for his constant interest and stimulating suggestions. I am also indebted to Professor L. O. Overholts of Pennsylvania State College for the specific identification of the fungus and to Dr. G. W. MacArthur for reading the manuscript.

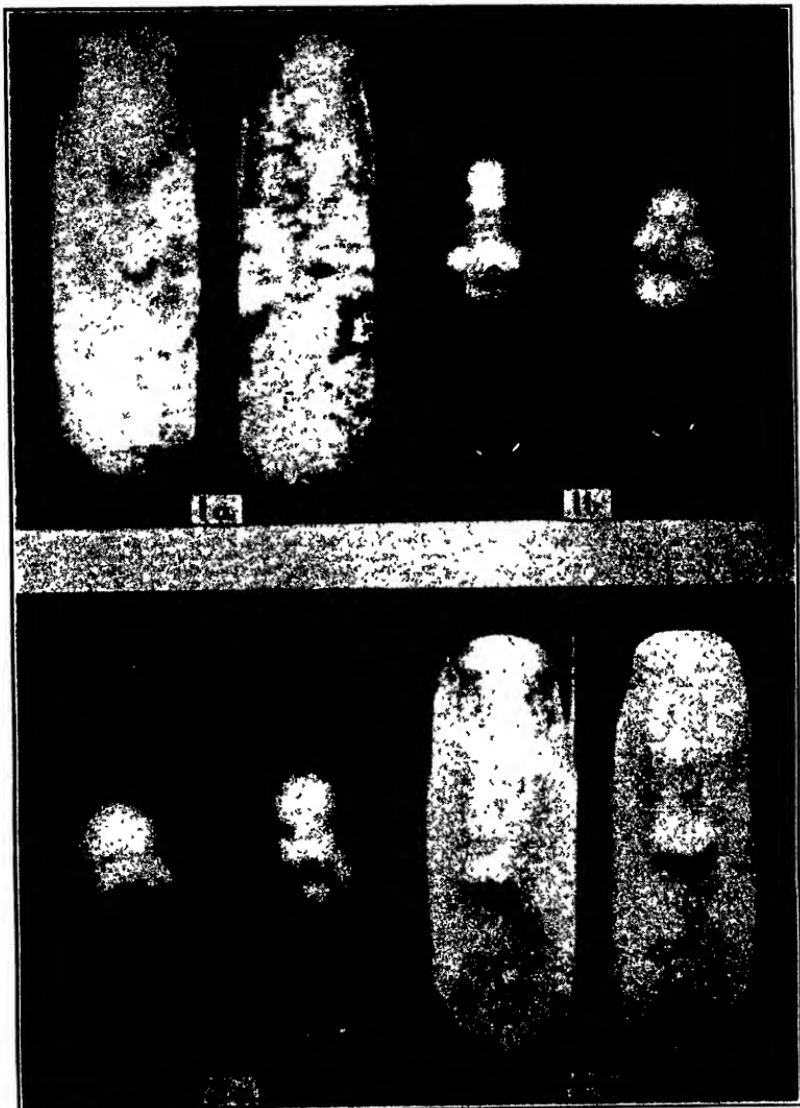
## SUMMARY

1. *Peniophora Allescheri* Bres. is genetically heterothallic.
2. Oedocephaloid conidiophores are produced on haploid and diploid mycelia. Conidia are uninucleate in both cases, those on the haploid mycelium repeating the haploid generation, those on the diploid mycelium giving rise to haploid mycelia of the two parental types, as determined by ability to react to form clamp-bearing mycelium.
3. A mutant in the haploid has appeared which differs from the normal haploid mycelium in type of growth and in its inability to produce conidia.
4. Mating this non-conidial mutant with a suitable normal haplont produces a diploid colony which reveals characters of both parents and bears conidia which when grown separately give rise to colonies, in equal numbers, having the characteristics of the two parents.

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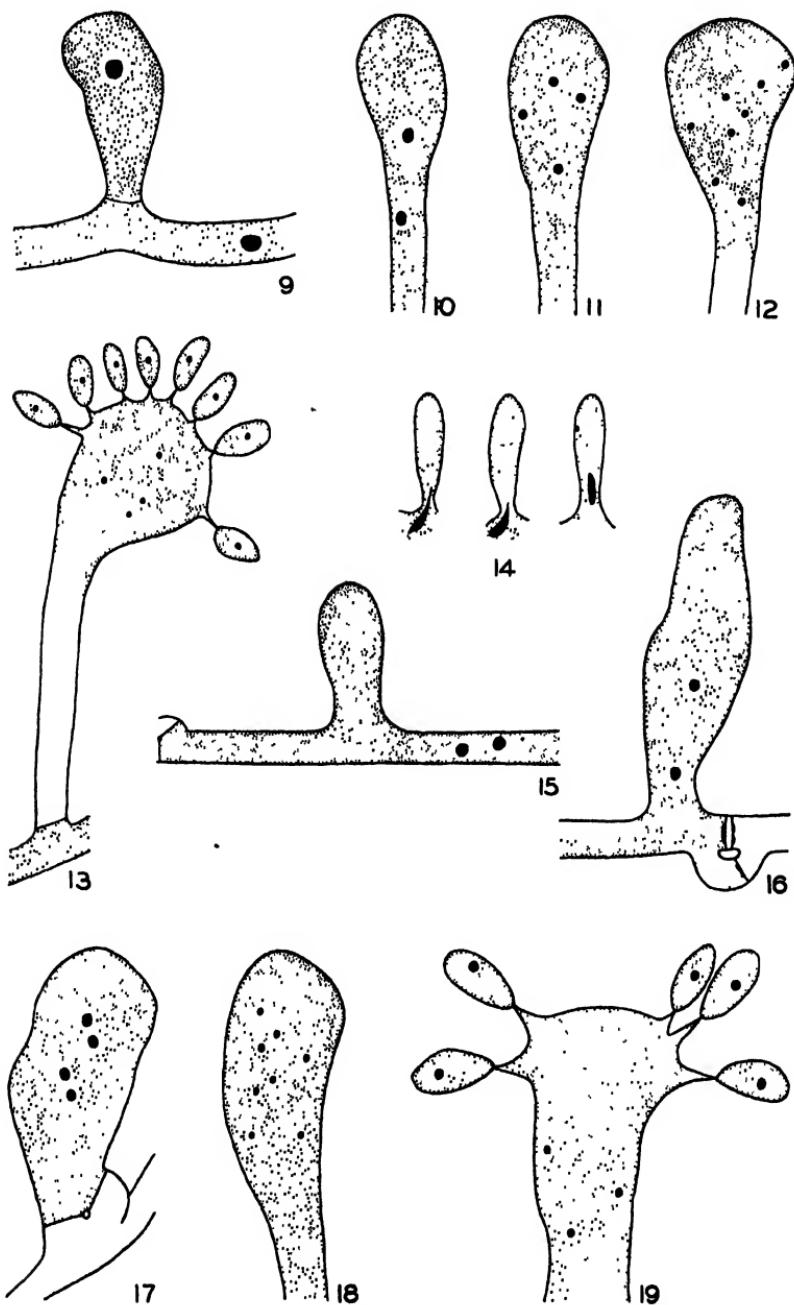
PENIOPHORA ALLESCHERI





PENIOPHORA ALLESCHERI





PENIOPHORA ALLESCHERI



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#### EXPLANATION OF PLATES 21-23

Fig. 1a, cultures of haploid non-conidial mutant 18 days after inoculation; 1b, cultures of normal haploid of the same age; 2a, cultures of normal diploid obtained by pairing 2 suitable conidial haplonts; 2b, cultures of hybrid obtained by pairing a normal conidial haplont and the non-conidial mutant; 3, haploid mycelium and young conidiophores.  $\times 360$ ; 4, the same field as that in fig. 3 taken twenty-four hours later.  $\times 360$ ; 5, conidiophores and conidia on the haploid mycelium.  $\times 360$ ; 6, clamp-bearing mycelium and conidiophores.  $\times 360$ ; 7, mycelium of the non-conidial mutant.  $\times 360$ ; 8, mycelium of the hybrid obtained by pairing a normal haplont and the non-conidial mutant; 9-13, stages in conidiophore development on the haploid mycelium.  $\times 2600$ ; 14, nuclei migrating into developing conidia.  $\times 2600$ ; 15-19, stages in conidiophore development on the diploid mycelium.  $\times 2600$ .

# BROWN ROT OF FRUITS AND ASSOCIATED DISEASES IN AUSTRALIA

T. H. HARRISON

(WITH PLATE 24 AND 2 TEXT FIGURES)

## PART II. AN INTERESTING DISCOMYCETE, *SCLEROTINIA AESTIVALIS* POLLOCK, OCCURRING ON MUMMIFIED FRUITS

### INTRODUCTION

In the spring of 1921, the author found apothecia of *Sclerotinia fructicola* (Wint.) Rehm., arising from apricot mummies in an orchard at Pennant Hills, near Sydney, New South Wales, Australia (3). In December of that year, he revisited the orchard and found, on several mummied apricots, numbers of small, pinkish, discoid apothecia. Some thousands of similar apothecia were later collected from mummified apricots and quinces, while numbers were also obtained from mummified apples, plums, peaches and pears. The constant association of these apothecia with fruits mummified by the brown rot fungus, led to the assumption that perhaps they were the apothecia of that fungus, an assumption not verified by microscopic examination.

Many features of this fungus, however, make it one of great interest to those working with the brown rot fungi, and also to mycologists generally, particularly to those engaged in teaching work. Some of these features are: (1) It is very easily obtained in pure culture from ascospore clouds; (2) it appears to have been recorded only once previously—by Pollock in Michigan, U. S. A., in 1909 (7); (3) crop after crop of apothecia may be obtained from the one mummied fruit, and this may extend for a period of up to 13 years; (4) in pedigree cultures obtained from single ascospores, it will, on artificial media in the laboratory, reproduce apothecia in a few months; (5) it has not produced a macroconidial stage, but microconidia are at times abundant; (6) it is normally a saprophyte, growing vigorously upon an unusually

wide range of nutritive media; (7) it appears to be capable of replacing the brown rot fungus in mummied fruits.

#### HISTORICAL

In 1909, Professor J. B. Pollock (7) of Ann Arbor, Michigan, U. S. A., recorded, as follows, a new species of *Sclerotinia*:

*Sclerotinia aestivalis* Pollock n. sp. Since the preceding part of this paper went to the printer a new species of *Sclerotinia* has been collected at Palmyra and Ann Arbor, Mich., first by Dr. L. H. Pennington, June 26, 1909, then by myself and several of my students through July and up to Aug. 9. It occurs on old mummied apples that have lain on the ground over winter. It is apparently not a parasite on either the apple or plum, since ascospores inoculated into ripe and unripe apples both sound and bruised did not develop a *Monilia* stage, nor cause a rapid rot of the apples. Similar negative results were obtained on inoculating the ascospores into plums. After several weeks in the moist chamber, a very slow rot on both takes place.

Apothecia from one to fifty on a single mummied apple; stalks short, rarely reaching 1 cm., not more than .5 mm. thick, dark brown and tapering below, lighter colored above; disc from 1-7 mm. in diameter, light reddish brown, flat when mature, radiating ridges and furrows below, not hairy but appearing almost velvety on outside; asci somewhat clavate or nearly cylindrical, short for the genus  $51-85 \times 6-8.5$  mostly  $68-75$  micromillimeters long, not turned blue by iodine, several from a common stalk when teased out; ascospores narrowly elliptical,  $6.4-11.9 \times 2-3.4$ , average size about  $8.5 \times 3$  micromillimeters.

This species differs from the other species of the genus occurring on fruits in the time at which the apothecia develop. They are usually produced in April or May, while this one occurs in midsummer, on account of which the species name *aestivalis* is chosen for it.

The conidial stage was searched for where the ascus fruits were found, but none was found. Attempts were made to get cultures of the ascospores as they were shot into the air, but no conidial stage developed. Whether there is no conidial stage, or whether the right conditions for its development have not been offered, is not at present ascertained.

This record was unsupported by illustrations, but specimens of the fungus were preserved. Pollock did not publish further details of this fungus.

In 1912, Demaree (1), while working in Professor J. B. S. Norton's laboratory, described a *Sclerotinia* which he had found on apples at College Park, Maryland, U. S. A., in November, 1911. His description agreed fairly well with that of Pollock's *S. aestivalis*, but Demaree did not know of this fungus. He was unable to produce growth from ascospores although he tried various media including fruit and cooked apples. He contrasted his fungus with Aderhold & Ruhland's *S. fructigena*, and supposed

the former to be the perfect stage of a fungus causing brown rot of apples in America.

Specimens of Demaree's fungus have not been preserved at College Park, Maryland.

J. B. S. Norton *et alii* (6), in 1923, made the following statement: "Demaree, in 1912, described a *Sclerotinia* on Maryland apples, which may be *Sclerotinia aestivalis* Pollock." In 1932, Norton saw specimens of *S. aestivalis* Pollock, and again expressed the opinion that this was probably the fungus which Demaree had. In the absence of specimens and illustrations of Demaree's fungus, however, it is impossible to be sure of this point, particularly as it is difficult to understand how anyone, who had *S. aestivalis* Pollock, could fail to produce growth from ascospores.

In 1928, the author (4, p. 140) made passing reference to this fungus. He stated that mummied apples collected near Sydney, early in 1922, "were producing apothecia of *S. aestivalis*," and that on 19th September, 1922, apothecia found on mummified apples, "consisted of the small flattened apothecia of *S. aestivalis*," and of *S. fructicola*. An illustration of the two apothecia on a mummied apple was published.

#### OCCURRENCE IN AUSTRALIA

ON APRICOTS. On December 25, 1921, beneath the apricot tree, under which apothecia of *S. fructicola* had been obtained in the previous September, the author found three mummied apricots, bearing a number of very small reddish brown, shortly stipitate apothecia. On the following day, an intensive search was made beneath apricot trees in an adjoining orchard. Fruits mummified, in the previous season, by the brown rot fungus were very abundant, especially in shallow depressions caused by the cultivation methods adopted. Under twenty trees selected for search, from 1 to 30 per cent of the mummied fruits each bore from 2 to 30 apothecia. Most of these were produced from the point where the half-buried mummied fruits made contact with the ground surface, but some fruits, in the moister spots, bore apothecia from fully exposed surfaces. The diameter of the disc varied from 0.5–6 mm., while the length of stipe

varied from 0.5–8 mm.—depending largely upon location. In some cases germination of the seeds in the mummied fruits was in progress, and one seedling, with apothecia of this fungus on the sclerotoid mass surrounding the seed, is illustrated in plate 24, fig. 4. One typical apothecium was found arising from the petiole of an apricot leaf, which was lying amongst a number of mummied fruits, most of which bore apothecia (PLATE 24, FIG. 5). It is very probable that the leaf was originally killed by the brown rot fungus, because twig blight was prevalent in the orchard during the previous season.

ON QUINCES. In the same orchard there were several quince trees, beneath which large numbers of apothecia were found on mummied quinces. The abundance of the apothecia is indicated by the fact that one group of 17 mummied quinces, or parts thereof, bore a total of 816, i.e. an average of 48 per quince. One quince bore 170 apothecia, most of which were mature. Excellent clouds of ascospores were expelled when this and other specimens were moved. Since 1921, the author has collected apothecia of *S. aestivalis* on quince mummies on many occasions, whenever required for class purposes. The mummied fruits apparently become thoroughly absorbed by the *S. aestivalis* mycelium, because they have an astonishing ability to produce many crops of ascocarps over an extended period. Some of the quinces bearing *S. aestivalis*, collected in December, 1921, have since been kept in his laboratory by Dr. W. L. Waterhouse, University of Sydney. Each year, at intervals dictated by class requirements, he has moistened the mummied fruits, which are set in soil in a seed pan, and he has not yet failed to obtain a crop of apothecia. Some quince mummies bearing *S. aestivalis* were collected by the author in February, 1928, and used for the production of several crops of apothecia at Hawkesbury College. An illustration of one such crop is shown in plate 24, fig. 1. Some of these mummied quinces were dried and taken to England in June, 1930. After being in an envelope for four months, the mummied fruits were placed in moist, sterile sand, and incubated, for a few days, at 25° C. A heavy crop of apothecia was produced for exhibition at the November meeting of the British Mycological Association in 1930. Since that time several further

crops have been produced, as required, from the same mummied fruits.

Another feature of importance is the longevity of the individual apothecia, some of which have continued to produce spores over a period of 5 weeks, in most cases growing appreciably meanwhile. Further, while many apothecia are mature, others are just commencing to emerge from the substratum. Individual mummied fruits have yielded apothecia continuously over a period of 4 months.

#### TIME OF DEVELOPMENT

The species name, *aestivalis*, was chosen by Pollock because he found the apothecia in midsummer, in contrast to the spring apothecial development of the Brown Rot fungus, *S. fructicola* (Wint.) Rehm. In New South Wales, the apothecia occur in greatest numbers in the field from December to March, but have also been noted in April, August, and September. In the laboratory at Sydney University, apothecia were developed continuously from December, 1921 to February, 1923. It would appear, however, that relatively warm temperatures are necessary for the development of the apothecia, and these are normally found at soil level, in weedy orchards, during midsummer in New South Wales. The optimum temperature for vegetative growth has been shown by experiment to be approximately 25° C., and this temperature has been used successfully to initiate apothecial development.

#### SIZE OF APOTHECIA

The size of the apothecia found on quinces varied greatly. It appeared to be partly dependent on the number of apothecia present on a mummied fruit. In one case where three apothecia occurred on a mummied fruit, the average diameter of the discs was 5.43 mm., while of 68 measured on a single fruit, the average diameter was 2.56 mm. The average diameter of the discs of 457 apothecia, collected in December, 1921, was 2.7 mm., but the range extended from 0.25 mm. to 10.5 mm. The size, however, of any individual apothecium is not constant, shrinkage taking place in a dry atmosphere, swelling in a moist one. Moreover, an apothecium may be mature and eject spores, when its diam-

eter is only 0.5 mm., and provided that moisture and suitable temperature are available, it may continue to grow to a diameter of 5-8 mm. It is clear, therefore, that the diameter of the disc in this fungus is so very variable that, alone, it is of little value for taxonomic purposes.

ON PLUM. On 14th January, 1922, following climatic conditions similar to those prevailing three weeks previously, 4 mummied plums, bearing apothecia, were found beneath a Japanese plum tree, in an orchard at Pennant Hills. One mummied plum bore 6, another 40, while the other two had intermediate numbers of apothecia present. The apothecia were mostly small, the largest having a diameter of 2 mm., while the longest stipe was also 2 mm. Conditions at this time were not very favourable for apothecial growth, although suitable for initiating their production.

ON PEAR. In April, 1921, Dr. W. L. Waterhouse placed a Keiffer's Hybrid pear, which had been rotted and mummified by the brown rot fungus, in his garden for observation. In February, 1922, he found a single apothecium of *S. aestivalis* arising from this mummied pear, which was brought to the University. After being placed on moist soil, and incubated for a period, this mummied pear gave a number of apothecia of this fungus, but none of *S. fruticola*.

ON APPLE. On 8th March, 1922, beneath a thick carpet of weeds such as *Fumaria officinalis* L., and *Plantago lanceolata* L., surrounding an apple tree in a moist part of an orchard at Pennant Hills, large numbers of apothecia of *S. aestivalis* were found. In some cases they arose from almost entire mummied apples, but in others they came from pieces of skin-like material, which ranged from the merest fragments to pieces representing half or more of the apple. In April, 1922, in another orchard in the same district, apothecia were found on mummied apples of the Trivett Seedling variety. In September of the same year, more apothecia were developed, in seed pans at the University, from mummified fruits collected in April (4, p. 140).

ON PEACH. In March, 1922, mummied peaches were collected at Pennant Hills, taken to the University, and there placed in soil. From one of these an apothecium, of the normal *S. aestivalis* type, was subsequently developed.

## DETAILS OF APOTHECIA

*Colour.* The colour varies considerably with the age of the apothecia, and with the amount of moisture present. Normally, however, the typical colour may be described as flesh pink, darkening appreciably to reddish-brown, and later to brown when drying and turning mealy with age. In terms of Ridgway's Standards (8), the range is from pale flesh pink to cornelian red, with the majority falling within the colour range between flesh colour and carrot red.

*Size.* As detailed in connection with quinces and apricots, the size of the disc is most variable. Pollock records that the diameter varied from 1 to 7 mm., while the author, with more material available, found the range greater, recording disc diameters ranging from 0.25 to 10.5 mm.

*Shape.* The first signs of the apothecia are small, rounded, pink coloured protuberances on the surface of the mummied fruit. These are gradually lifted above the surface by elongation of the stipes. The end of the stipe is flattened soon after emergence, but quickly becomes concave, to form the apothecium. At first crateriform, the apothecium becomes campanulate, often flattened and, in some cases, convex. The margin is obtuse, glabrous, and usually remains entire.

*Stipe.* The apothecia are positively phototropic, hence the length of the stipes is governed largely by the point of origin of the apothecia. Of 105 apothecia arising from mummied quinces, the average stipe length was 3.445 mm., but in this set the range was from 0.25 to 12.5 mm. The greatest stipe length noted by the author was 27 mm.

Usually the stipe is unbranched, but several examples of two and three apothecia on a branching stipe have been noticed (PLATE 24, FIG. 6). On several occasions, apothecia have been the point of origin of further apothecia—the stipes arising from the centre of the old discs. One such interesting specimen had a branched stipe bearing two apothecia. From each of these apothecia, three further apothecia were produced, making eight in all from a single point of origin. Another unbranched stipe bore a medium sized apothecium, from which seven stipes were sent up to bear small apothecia.

The colour of the stipe ranges from the black of the substratum in which it originates to the pink colour of the disc. The stipe is usually thin, gradually expanding to form the base of the disc. Rhizoids are absent.

*Nature of Substratum.* A point of considerable taxonomic significance in the *Sclerotiniaeae* is the nature of the material from which the apothecia originate. In this case, the apothecia arise from fruits mummied by the brown rot fungus, *i.e.* from a structure which, consisting of fungus and incorporated host tissue, has been defined by Honey (5) as a pseudosclerotium. This pseudosclerotium, however, is all host tissue for the fungus, *S. aestivalis*. No sclerotia were found in association with the apothecia, either on the mummied fruits or on the apricot petiole. In pure culture, however, flattened sclerotoid bodies, from which apothecia develop, are produced in the medium.

*The Ascii.* The shape of the ascus is indicated in text figure A. The size ranged from  $56.6-81.7 \mu \times 4.8-7.7 \mu$  for 200 ascus measured. Size depends considerably upon the time, in development, at which the measurements are made. In this case, ascus showing mature spores in a distichous arrangement were measured.

Spores are arranged at first in a monostichous manner, but towards maturity they move towards the top end of the ascus, overlapping one another and eventually becoming distichous just prior to expulsion. Spores are ejected forcibly from the ascus when the pressure of the surrounding atmosphere is reduced, producing a conspicuous cloud, which has been photographed.

The top end of the ascus is rounded until the ascospores assume the distichous arrangement, when, just prior to ejection of spores, it becomes flattened. When the spores have been ejected, the end is square and somewhat frayed. The pore stains blue with iodine.

The hymenium contains ascus at all stages of development at any one period (TEXT FIG. A, FIG. 5). This probably explains the unusually extended period during which ascospores may be ejected from a given apothecium.

*Paraphyses* vary in size, being sometimes shorter but usually slightly longer than the ascus. They are usually 2-3-septate, hyaline, filiform, single or branched, only slightly swollen at the apex.

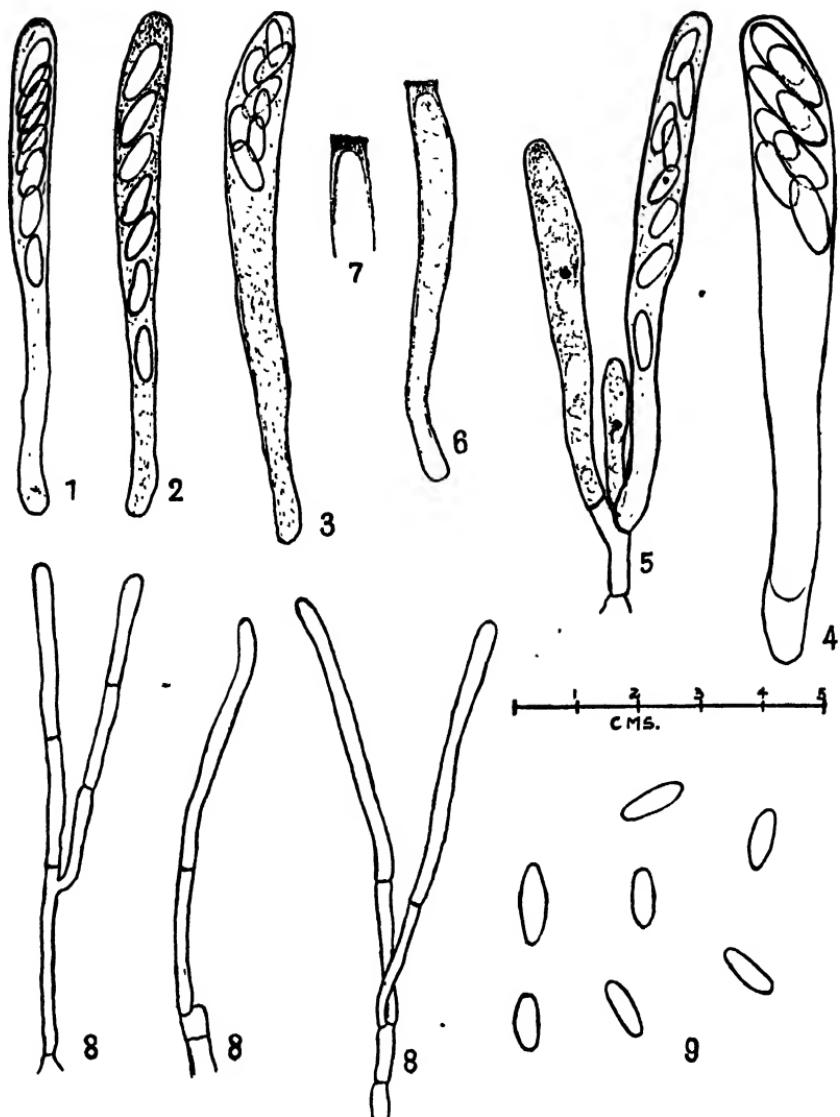


FIG. A. 1-4, asci showing various arrangements of ascospores; 1 sub-seriate, 2 monostichous, 3 and 4 distichous; 5, portion of hymenium, showing asci in various stages of development; 6, ascus from which spores have been ejected. Note frayed square end; 7, ascus as in 6, stained with iodine, showing central dark blue pipe, indicating the position of the pore; 8, paraphyses—single and branched; 9, a group of ascospores. All drawn with the aid of a camera lucida. Magnification, in original plate, 1000 except 4 which is  $\times 1400$ . The reduction for this text figure is indicated by the centimetre scale included.

*Ascospores.* The spores are hyaline, elongated, ellipsoidal, with sharply rounded ends (TEXT FIG. A, FIG. 9). They have no conspicuous oil spots or vacuoles. The spores measured 6–10  $\mu$   $\times$  2–3.5  $\mu$ , with an average of 8.56  $\times$  2.97  $\mu$  for 300 spores measured.

Germination of the ascospores takes place rapidly in distilled water, or upon the surface of many nutritive media. At 25° C., in 2 per cent dextrose or in prune juice decoction, it is very rapid. Germination from both ends of the spore is usual, although one germ tube is generally somewhat earlier than the other. The germ tube is surrounded by a gelatinous sheath, easily shown in Indian ink or gentian violet. Branching occurs freely, and, while not regular, is always lateral, and usually at an angle greater than 45° (TEXT FIG. B).

Septa soon appear and the cells vary in size. Some hyphae are constricted at the septa, others not. Adjacent cells in a hypha may show the two conditions, while the diameter of the hyphae is also most variable. It appears, therefore, that these features of the germ tube and vegetative hyphae have no taxonomic significance for this fungus.

*Method of Obtaining Single Spore Cultures.* Ascospores, ejected by mature apothecia, were collected on clear 2 per cent plain agar, in inverted petri dishes. The dish was held in such a way that a well distributed seeding of ascospores was obtained on the surface of the cooled agar. The closed petri dish was left at laboratory temperature for a period of 14–24 hours, dependent on temperature. The closed petri dish was inverted on the stage of the microscope, and with the low power objective (16 mm.), the surface of the agar was located and searched for sporelings. The position of well isolated sporelings was marked by a drop of Indian ink, the centre of which was immediately removed by a small pointed piece of blotting or filter paper. This left the sporelings immediately above the centre of a ring of Indian ink. When as many sporelings as desired were located in this way, the petri dish lid was removed and with a special platinum needle, the agar, containing the desired sporelings, was cut away from the surrounding agar, but not removed at this stage. The ink markings were then removed from the bottom of the petri dish,

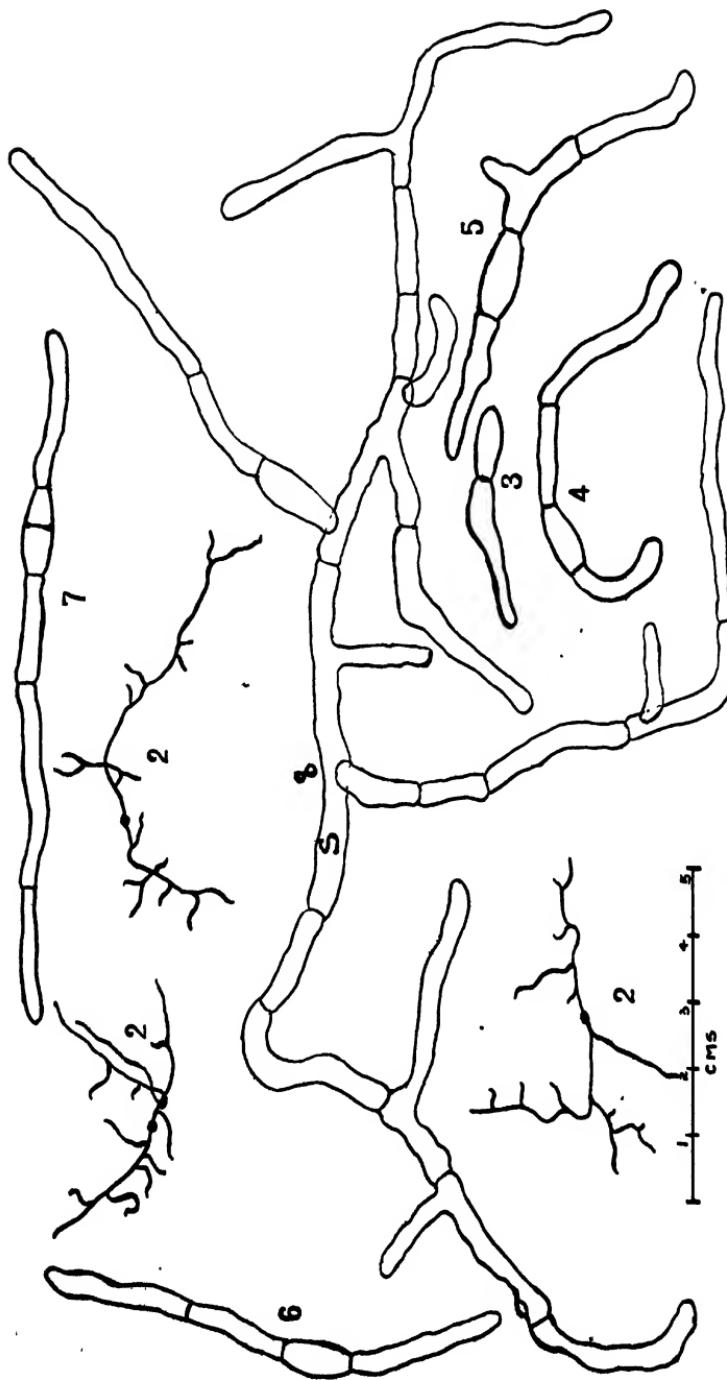


FIG. B. 2, diagrammatic tracing of sporelings on dextrose 2%, the position of the spore being indicated by the dots; 3-7. germination, in prune juice decoction, at room temperature at Sydney, January, 1922. All drawn 20-22 hours after planting; 8, sporeling on 2% dextrose agar, held for 24 hours at 25° C. and for 18 hours at 15° C. All drawn with the aid of a camera lucida. The magnification for the original plate is 1000 except No. 2 where it is  $\times 200$ . The variation from this is indicated by the scale incorporated in the plate.

and the fact that the sporelings were uncontaminated, and completely isolated in an island of agar, was verified by microscopic observation. With a 10 X eyepiece, a 16 mm. objective was usually sufficient, but, if in doubt, an objective such as 4 mm. was used. The isolated sporelings were then removed on their platforms of agar to the edge of nutrient slopes, and again the purity of the culture was verified by microscopic observation. The growth was then watched carefully, so that no chance of contamination occurred.

#### GROWTH ON ARTIFICIAL MEDIA

*Sclerotinia aestivalis* has been grown under observation upon the following media:

*Nutrient agar* in tubes and petri dishes: potato dextrose, maize meal, prune juice 2 per cent, malt extract, mummy extract, oatmeal, Brown's a, Coon's, Richard's and 2 per cent plain agar; *sterilised plugs or portions* of the following: potato, prune, pear, apple, French bean pod, radish, parsnip, carrot; *stems* of tomato, castor oil plant, maize, lucerne; *petioles* of celery, cowpeas, silver beet, rhubarb; *crushed grains* of maize, wheat, barley, oats, rye, rice, soybeans; *leaves and bark* of the Turpentine (*Tristania laurifolia*); and *shoots* of eschalot.

In all cases portions of the above were placed in tubes, and a few ccs. of distilled water added. They were sterilised in the autoclave at 15 lb. pressure for 20 minutes. After inoculation, the tubes were incubated at 25° C., and detailed observations made at intervals.

It is a tribute to the tolerance of the fungus to changes in the nutritive medium, that it was able to grow on all the above materials. In all cases there was produced a surface and sub-aerial mycelial growth, white and flocculent at times, but devoid of a macroconidial stage. Usually there was formed beneath the surface mycelium, a dark brown to black sclerotial crust, which may extend to cover the exposed area of the medium. Considerable nigrescence of the medium, to varying depths, takes place.

*Macroconidial Stage.* The author has tried many times to induce production of an imperfect spore stage, but, so far, all efforts have failed. This fungus has not only been grown on a

wide range of media, but has been subjected to rapid changes in environment, and also to starvation methods, which have proved successful in inducing conidial development in other fungi. Every likely culture has been examined microscopically. In some preparations, hyphae have been noted with very numerous septa, giving the appearance of chains of immature chlamydospores, but none of these has been observed to develop further, and to abstract.

It is possible that this fungus is devoid of a macroconidial stage—a condition in agreement with Whetzel's contention that the fungus is a *Ciboria*.

*Microconidia*, agreeing in shape, size and method of production, with those described for other members of the *Sclerotiniaeae*, have been observed.

*Return of Apothecia in Pure Culture.* In May, 1922, a monosporic isolate, obtained from an apothecium on quince, was subcultured on malt extract agar in a petri dish. This culture was kept at room temperature, in a laboratory at the Agriculture School, Sydney University. On August 16, i.e. approximately 15 weeks later, one of the sclerotiod bodies, which had been formed in the medium, bore a mature apothecium. Further apothecia were later produced from this and other sclerotiod bodies (PLATE 24, FIG. 7).

In December, 1930, the author supplied Mr. F. T. Brooks of Cambridge with a single ascospore culture, isolated in London. Brooks subsequently reported that in a large petri dish, 4 months after subculturing onto P.D.A., he obtained a large crop of apothecia. The apothecia in this case, too, arose from thick, black, flattened, sclerotiod bodies, developed at intervals in the medium.

A monosporic culture of *S. aestivialis* was also supplied to Professor H. H. Whetzel, at Cornell, who obtained abundant crops of apothecia in artificial media.

While it is possible that the monosporic culture, from which apothecia arose in Sydney in 1922, may have become "contaminated" with another "strain" of *S. aestivialis*, it is impossible in the two other cases. A monosporic culture, supplied by the author in each case, was the only material of *S. aestivialis* avail-

able. This evidence shows clearly that *S. aestivialis* is homothallic.

#### REACTION ON FRUITS

In February, 1923, selected Trivett seedling apples were surface sterilised with alcohol, and inoculated with an active culture of *S. aestivialis*, in comparison with the *S. fructicola* (Wint.) Rehm., and *S. sclerotiorum* (Lib.) De Bary. Detailed observations were made over a period of 5 weeks, the apples being kept under bell jars in the laboratory. It was shown that at the end of the period, *S. aestivialis* had extended only through an area of 6 mm. on either side of the wedge of apple, cut out for inoculation purposes.

The observation was verified by a separate experiment in 1923 and on several occasions in London in 1931. *S. aestivialis* can produce a very slow dark brown rot in apples, but is not capable of causing complete rotting and mummification of fruits. It could not have been responsible, therefore, for the mummification of the fruits on which it was found in such abundance.

Further work is contemplated on the relationship of *S. fructicola* and *S. aestivialis*, in view of the almost constant association of the latter with fruits mummified by the former. That the association is actual may be illustrated by the following example. In 1930, Dr. W. L. Waterhouse, of Sydney, forwarded to London 13 quinces which he knew to be mummified by the brown rot fungus. From 8 of them, in England, the author developed apothecia of *S. aestivialis*.

*Contrast with S. sclerotiorum (Lib.) De Bary.* It has been suggested that the fungus described above was merely *S. sclerotiorum* (Lib.) De Bary. On many occasions, the author has isolated this fungus from infected plant tissue in Australia, and has grown it in culture on a large number of media, as well as on various fruits. He has also developed the apothecia to maturity in pure culture. Not only is this fungus strikingly different in cultural behaviour and parasitic ability, but morphological features of taxonomic significance are different from those in *S. aestivialis*, e.g. the asci of *S. sclerotiorum* are twice as long as those of *S. aestivialis*. The author also had *S. aestivialis* growing under comparable conditions with many strains of *S.*

*sclerotiorum* in England during 1931. He also saw much of Ghamrawi's comparative work upon *S. sclerotiorum* and *S. Trifoliorum* Erik. The author is convinced that *S. aestivalis* Pollock is a species distinct from both *S. sclerotiorum* (Lib.) De Bary and *S. Trifoliorum* Erik.

#### THE TAXONOMIC POSITION

Through the courtesy of Dr. C. L. Shear, of the U. S. D. A., some of the type material, supplied by Professor J. B. Pollock, has been studied. In all morphological details, this material agreed with that in the possession of the author, and with Pollock's description of it.

A study of the foregoing pages will show that the author, by virtue of the larger quantity of material available, has found a greater range in certain features than is quoted by Pollock, but in essential morphologic details, Pollock's description remains an excellent one of the fungus found in Australia. The Australian fungus is, therefore, cospecific with that described by Pollock in 1909.

#### NOMENCLATURE

The historical review shows clearly that the only name proposed for this fungus is *Sclerotinia aestivalis* Pollock. It is properly published, with an adequate description, and, therefore, the specific name *aestivalis* must stand irrespective of the suggestion to follow.

The close affinities existing between *Sclerotinia*, *Ciboria*, and others of the family *Helotiaceae* are generally recognised, but Fuckel's original generic criteria have proven unsatisfactory. Professor Whetzel has found it necessary to revise and define more clearly the generic concepts in the sub-family *Sclerotiniaceae*. In March, 1932, Whetzel, after a study of the author's material of *S. aestivalis*, expressed the view that the fungus should be included in the genus *Ciboria*, rather than in *Sclerotinia*, because the sclerotoid bodies were produced *in* the medium and not free from it, as is the case with a typical *Sclerotinia*. It has been stressed above that the apothecia in nature arise directly from the host tissue.

In August, 1932, monospore cultures of this fungus were forwarded to Whetzel, who, on December 15, 1932, wrote to the author as follows: "I am pleased to report that S 961, (a monospore culture of *S. aestivalis*) produced apothecia in abundance for me. It is clearly a *Ciboria*, and a most typical one. In your future publications on this form you need have no hesitation in placing it in the genus *Ciboria*."

The author feels justified in accepting Whetzel's authority for the change of the generic name.

The new combination, *Ciboria aestivalis* (Pollock) Whetzel, is proposed for the fungus described in this paper.

#### SUMMARY

1. An interesting Discomycete associated with fruits mummified by the brown rot fungus, *S. fructicola* (Wint.) Rehm., is recorded for the first time in Australia.
2. Taxonomic details are presented to show that it agrees with a fungus described as *Sclerotinia aestivalis* by Pollock in Michigan, U. S. A., in 1909.
3. In New South Wales, it has been found on mummied apples, apricots, peaches, pears, plums and quinces. Details of its occurrence on each host are given.
4. The apothecia are very abundant. On a quince mummy, 170 apothecia were present at the one period. Many crops are produced from the one mummied quince. Some have given apothecia each year for 13 years.
5. The method adopted by the author for isolating pedigree cultures from single ascospores is described. The fungus grows readily on an extensive range of artificial media.
6. No macroconidial stage has been found, despite careful searches on many media, kept under varying conditions calculated to induce sporulation.
7. Apothecia have been obtained in pedigree cultures on artificial media, held in laboratories in Australia, England and America, for 3-4 months.
8. For practical purposes it may be regarded as a saprophyte, although it will cause a very slow rotting of apples, when held for long periods under favourable conditions for the growth of

the fungus. It may be parasitic upon the pseudosclerotium of the brown rot fungus, *S. fructicola* (Wint.) Rehm.

9. On the authority of Professor H. H. Whetzel of Cornell University the fungus is transferred from the genus *Sclerotinia* to *Ciboria*.

#### ACKNOWLEDGMENTS

It is a pleasure to acknowledge indebtedness to Dr. W. L. Waterhouse for inspiration and guidance during the early part of the work and for the photographs; to Dr. C. L. Shear for securing the type material; and to Professor H. H. Whetzel for helpful suggestions.

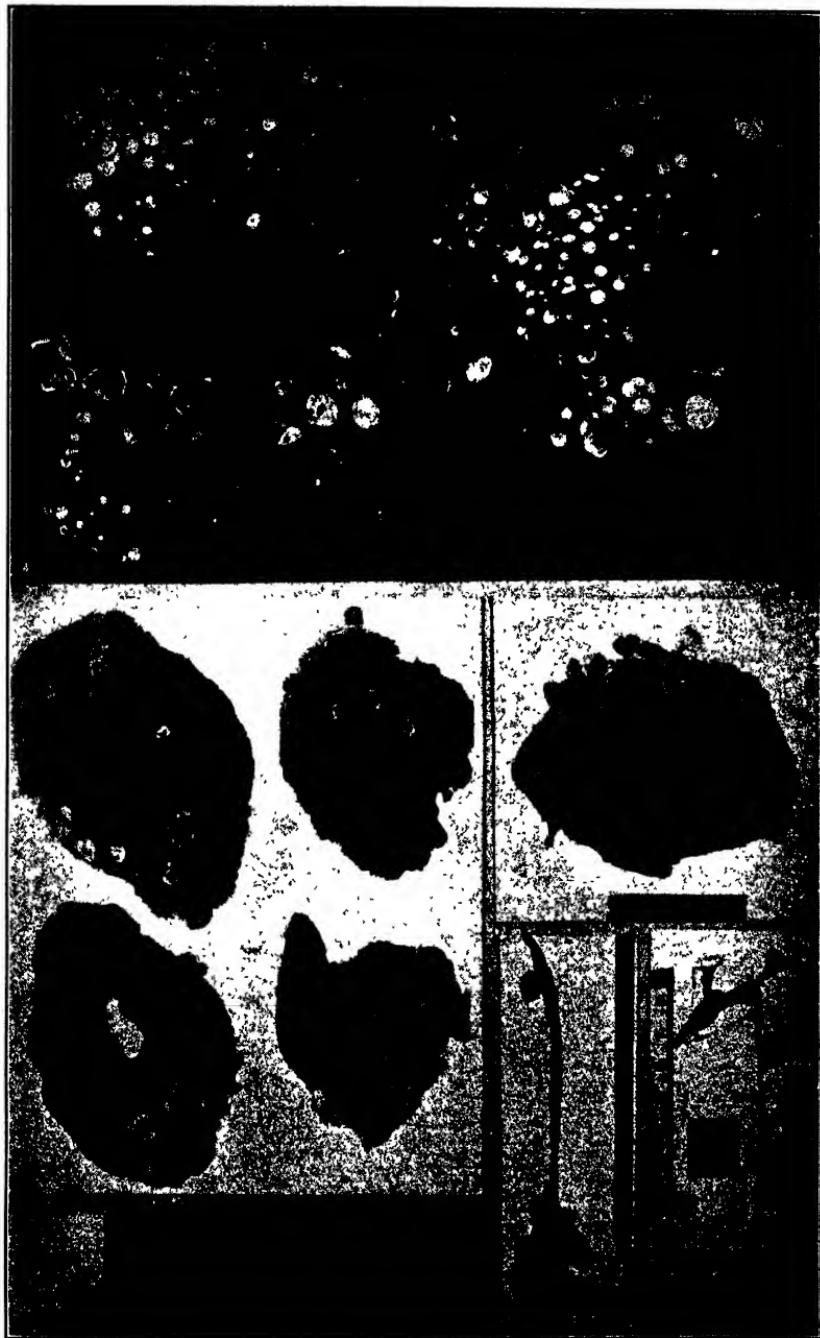
HAWKESBURY AGRICULTURAL COLLEGE,  
RICHMOND, NEW SOUTH WALES,  
AUSTRALIA

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#### EXPLANATION OF PLATE 24

*Sclerotinia aestivalis* Pollock. Fig. 1, crop of apothecia produced at Hawkesbury College, N. S. W., from mummied quinces collected at Pennant Hills, N. S. W., in February, 1928.  $\times 1.25$ ; 2, apothecia from mummied quinces, one of which bore 170 specimens.  $\times 0.92$ ; 3, cluster of apothecia on mummied apricot.  $\times 1.25$ ; 4, apricot seedling arising from seed enclosed by a black pseudosclerotium bearing apothecia of *S. aestivalis*.  $\times 0.36$ ; 5, apothecium arising from the petiole of an apricot leaf.  $\times 1.5$ ; 6, three apothecia arising from a single stipe.  $\times 1.25$ ; 7, apothecia on petri dish culture of M. E. A. Note the dark sclerotoid bodies which were produced in the medium. Natural size. All material illustrated in fig. 2-6 was collected at Pennant Hills, N. S. W., in December, 1921. Photographs by Dr. W. L. Waterhouse.



*SCLEROTINIA AESTIVALIS*



# EXPERIMENTS WITH HETEROECIOUS RUSTS<sup>1</sup>

GEORGE B. CUMMINS

## AECIDIUM HYDNOIDEUM BERK. & CURT.

Field observations made by the writer near Lafayette, Ind., in 1931 indicated that this conspicuous rust on *Dirca palustris* might form its alternate stage on *Carex*. June 3, 1932 the aecia were collected and the spores used to inoculate *Carex pennsylvanica*. A scattered production of uredia was evident June 18; no telia developed. Attempts to infect the *Dirca* with teliospores found associated with it in the field have been unsuccessful, the plants never remaining alive after removal from the field to the greenhouse.

The urediospores obtained from this culture agreed with those of *Puccinia extensicola* and formed the basis on which Arthur in the "Manual of the Rusts in United States and Canada," page 200, created the variety *P. extensicola hydnoidea*.

## PUCCINIA CARICIS-STRICTAE DIET.

Through the kindness of Mr. Roy Latham of Orient, Long Island, N. Y., the writer was supplied with *Carex stricta* collected January 31, 1933 and bearing amphispores and teliospores of *P. Caricis-strictae*. The teliospores germinated freely in April and were used on April 3, 1933 to inoculate young plants of *Urtica dioica*. An abundant infection developed with pycnia appearing April 9 followed by aecia on April 17. The same telial material was again used on April 12, 1933 and gave equally positive results on 12 pots of *Urtica dioica*, the pycnia appearing April 17 and the aecia April 25.

A comparison of this rust with the common *Urtica-Carex* rust, *Puccinia Caricis*, proved the two to be similar. Arthur (l.c. p. 208) used this culture as the basis for his erection of the variety *P. Caricis Caricis-strictae*.

<sup>1</sup> Contribution from the Botany Department, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

This culture is especially interesting in that it indicates that so-called amphisporic species may be only variants of rusts not producing amphispores in the normal life-cycle. If this is true it can be predicted that *P. atrofusca* will produce aecia on species of *Artemisia*, the chief aecial hosts of *P. universalis* (*Dicaeoma Dracunculi*). These two forms were united by Arthur (*l.c.* p. 205) under the name *P. atrofusca*. In like manner the aecia of *P. vexans* may be expected to occur on the Asclepiadaceae and to agree with those of *P. Bartholomaei*. In attempts to culture *P. vexans* I have never been successful in germinating the few teliospores which have been found. This rust ordinarily is found only in the amphisporic condition.

PUCCINIA SMILACIS SCHW. AND AECIDIUM APOCYNI SCHW.

This species has long been considered to be autoecious but field observations communicated by Mr. Roy Latham indicated that this was not true. Although uredia and telia commonly occurred on Long Island he never observed the development of aecia on the same host, *Smilax rotundifolia*.

Teliospores on *S. rotundifolia* collected at Mattituck, L. I., N. Y., by Mr. Latham were used in an unsuccessful attempt, made June 16, 1933, to infect plants of the same species at Lafayette, Ind. Mr. Latham then suggested that *Aecidium Apocyni* might belong to the *Smilax*-rust. He kindly furnished old aecia of *A. Apocyni* collected at Mattituck in association with *P. Smilacis*, August 3, 1933. No germination was secured on slides and no infection resulted from an inoculation of *Smilax rotundifolia*.

Mr. Latham again collected telia on *S. rotundifolia* at Orient, Long Island, December 26, 1933 and kindly sent me a supply which were over-wintered at Lafayette. Inoculation of plants of *Apocynum cannabinum*, also furnished by Mr. Latham, May 15, 1934 resulted in an abundant infection, the pycnia appearing May 23 followed by aecia on and after June 3.

The only known collections of *Aecidium Smilacis* were made in North and South Carolina. This aecial form can no longer be considered to belong to *P. Smilacis*. That *A. Smilacis* may belong to *Puccinia Arundinariae* was suggested by Arthur (*l.c.*

p. 383). Through the kindness of Mr. M. A. Rice of Clemson College, S. C., I have been able to over-winter teliospores of the *Arundinaria*-rust at Lafayette but have not been able to germinate the spores and cannot, therefore, report on the correctness of the above suggestion.

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LAFAYETTE, INDIANA

## NOTES AND BRIEF ARTICLES

### BULLER'S RESEARCHES ON FUNGI

Volume VI of the above work has recently appeared and exhibits the same painstaking care and patience which have characterized previous volumes.

Part I is devoted to the *Pilobolus* gun and its projectile. It is here shown that *Pilobolus Kleinii* and *P. longipes* can both shoot their sporangia vertically to a height exceeding six feet or a horizontal distance of eight feet. The mechanism of this discharge is fully discussed. The sporangium has the form of a plano-convex lens and in landing nearly always does so with the flat gelatinous side next to the object on which it lands. The sporangia thus tightly adhering to blades of grass have often been mistaken by mycologists (including the writer) for fruiting bodies of parasitic fungi. The sporangia thus placed are readily devoured by herbivorous animals and on release germinate and grow. Chapter three of part I is devoted to *Pilobolus umbonatus*, described as a new species on page 178 of this volume.

Part II consists of a description of the phenomenon of puffing in *Sarcoscypha proteana* and other discomycetes. In many of the cup-fungi the spores are shot to a distance of several centimeters above the hymenium and are then apparently dependent upon currents of air for their further distribution. The puffing is often accompanied by an audible sound as has been frequently noted.

Part III consists of a description of *Omphalia flava* and *Sclerotium coffeicola*, two fungi parasitic on the leaves of coffee. The entire volume consists of 513 pages and 231 illustrations. Like previous volumes it is published by Longmans, Green and Company. F. J. SEAVER

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### THE RECOVERY OF A PATHOGENIC FUNGUS FROM LEUCOCYTES

During an investigation conducted to determine the cause of a nasal granuloma<sup>1</sup> in cattle in Louisiana a fungus was isolated

<sup>1</sup> Nasal granuloma in cattle in Louisiana. N. Am. Vet. 15: No. 9. 1934.

which grew readily and fruited abundantly on Sabouraud medium at blood heat or more slowly at ordinary room temperature.

Guinea-pigs were inoculated with cultures of this organism, and after 7-10 days were found to have developed mycosis at the point of inoculation, in the omentum and the liver. Leucocytes from these lesions were found to contain 1-3 or more irregularly spherical, refractive bodies which in culture medium sent out a germ tube which became septate and branched. From these structures fruiting cultures were developed in 48 hours.

A paper on the morphology and systematic position of this fungus is in preparation.

VERA K. CHARLES

BUREAU OF PLANT INDUSTRY

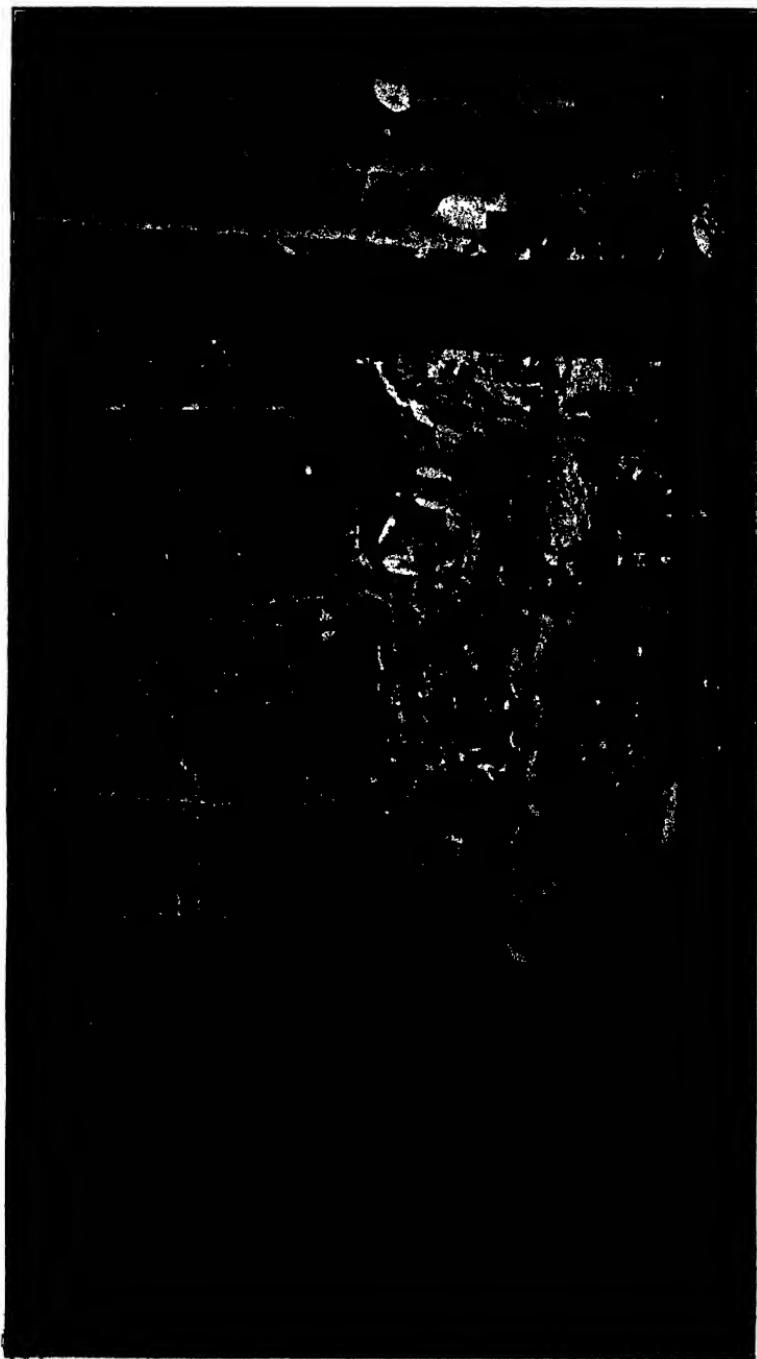
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### The Mycological Society of America

#### SUMMER FORAY

The second annual Foray of the Mycological Society of America was held at Seventh Lake, near Inlet, New York, August 21st-24th. The meeting was well attended by about forty-five mycologists and pathologists, twenty-seven of whom were members of the Mycological Society. Four of the present officers and members of the council were present, and among others such well known figures as Drs. R. A. Harper, C. L. Shear, John Dearness and C. Thom. Non-mycological members of families and guests brought the total attendance to seventy.

The Headquarters for the Foray was at the summer camp of Dr. F. C. Stewart on the north shore of Seventh Lake. Some of the visitors were accommodated at the camp, the majority, however, in summer hotels in the vicinity. The boat house at the camp was fitted for a laboratory, including microscopes and a good working library—part of which was loaned from the Department of Plant Pathology at Cornell and part from the Geneva Experiment Station. Facilities for drying mushrooms and pressed material were provided in a tent nearby. Another laboratory was provided at Johnston's camp near Sixth Lake, where facilities for handling parasitic fungi were provided under the leadership of Professors Whetzel and Welch of Cornell



Summer foray; picnic on Seventh Lake.

University. Lunch and dinner were provided each day for the entire party by Mrs. Stewart and daughters in an eminently successful manner.

Collecting on the part of those interested in the Basidiomycetes was done mostly in the immediate vicinity of Seventh Lake under the leadership of Dr. Stewart. Those interested in the parasitic fungi, under the leadership of Professor H. H. Whetzel, visited several outlying localities, thus dividing the party on several occasions into two groups referred to as the "fleshies" and the "parasites." On Friday, following a trip to Bug Lake by the "fleshies" and to Blue Mountain by the "parasites," the two groups came together at noon at a camp site between Seventh and Eighth Lakes for a most excellent picnic lunch provided by Mrs. Stewart and helpers. Following this lunch the party was entertained most successfully by Mr. Hoffmaster with a clever sleight of hand performance.

While the collecting could be considered only fair on account of the rather dry weather preceding the meeting, an abundance of material was obtained in all groups. Perhaps the most notable collection on which a report is available at this writing was that made by Mr. Robert Hagelstein, honorary curator for Myxomycetes at the New York Botanical Garden, who obtained 76 species and 3 varieties of Myxomycetes. From several dung collections made at the time of the Foray, Dr. R. F. Cain, of the University of Toronto, has since obtained the development in moist chamber cultures of 29 species of "Sordariaceae" including 8 European species previously recognized in North America only in Ontario, and 7 of the species described as new in his recent paper on this group. Mr. J. L. Lowe collected a large number of Lichens but no report is yet available. Drs. L. O. Overholts, A. H. Smith and W. H. Snell among others made extensive collections of the higher Basidiomycetes. A partial list including some of the less common fungi which were collected is given below with the name of the person or laboratory sponsoring the report.

The unqualified success of the Foray was due in no small measure to the untiring efforts of Dr. Stewart, who, with the able assistance of our Secretary-Treasurer, Dr. H. M. Fitzpatrick and others from the Cornell Laboratory, spared no effort to

make the occasion one to be long remembered by those who were fortunate enough to be able to attend. Special mention is due Mrs. Stewart, who was responsible for the most excellent meals.

#### NOTEWORTHY COLLECTIONS

Ascomycetes: *Adelopus balsamicola* (Peck) Thiess., on *Abies balsamea*, coll. Cornell; *Bifusella Faullii* Darker, on *Abies balsamea*, coll. Darker; *Diaporthe Pruni* Ellis & Ev., coll. Wehmeyer; *Diaporthe Macounii* Ellis & Ev., coll. Wehmeyer; *Enchnoa lanata* (Fries) Sacc., coll. Wehmeyer; *Geoglossum alveolatum* Durand, coll. Jackson; *Gloeoglossum affine* Durand, coll. Jackson; *Hypoderma rufilabrum* (Berk. & Curt.) Duby., on *Acer spicatum*, coll. Jackson & Darker; *Hypodermella nervata* Darker, on *Abies balsamea*, coll. Darker; *Lasiosphaeria muscicola* DeNot., coll. Cornell; *Lophodermium filiforme* Darker, on *Picea rubra*, coll. Darker; *Mollisia Iridis* (Rehm) Sacc., coll. Cornell; *Pezicula minuta* Peck, on *Viburnum*, coll. Wehmeyer; *Trichoglossum Walteri* (Berk.) Durand, coll. Jackson; *Ascocalyx Abietis* Nau-mov., on *Abies balsamea*, coll. Jackson; *Lachnum myricaceum* (Peck) Sacc. (*Peziza myricacea* Peck) on *Myrica gale*, coll. Shear; *Bolina atrovirens* Ellis & Ev., on *Fagus*, coll. Shear.

Thelephoraceae: *Aleurodiscus Farlowii* Burt, on *Abies balsamea*, coll. Jackson; *Corticium botryoideum* Overh., coll. Overholts; *Peniophora viticola* (Schw.) H. & L., coll. Overholts.

Clavariaceae: *Clavaria subfalcata* Atk., coll. Jackson; *Physalacria inflata* (Schw.) Peck, coll. Overholts.

Polyporaceae & Boletaceae: *Boletus placidus* Bon., coll. Snell; *Boletus leucophaeus* Pers., coll. Snell; *Boletinus spectabilis* Peck, coll. Snell; *Polyporus semisupinus* Berk. & Curt., coll. Snell; *Polyporus benzoinus* (Wahl) Fries, coll. Snell; *Trametes mollis* (Sommerf.) Fries, coll. Overholts.

Agaricaceae: *Crepidotus stipitatus* Kauff., coll. Smith; *Hypholoma holanigerum* Atk., coll. Smith; *Lacterius aspideoides* Burl., coll. Smith & Snell; *Mycena margaritispora* Lange, coll. Smith; *Mycena subviscida* Kauff. & Sm., coll. Smith; *Pholiota intermedia* Smith, coll. Smith; *Pluteus coccineus* (Mass.) Beard., coll. Smith.

Uredinales: *Calyptospora goeppertiae* Kuhn, III on *Vaccinium pensylvanicum*, coll. Cornell; *Chrysomyxa Cassandrae*

(Peck & Curt.) Tranz., I on *Picea mariana*, coll. Cornell; *Glomerularia Lonicerae* (Peck) Dearness & House, on *Lonicera canadensis*, coll. Cornell; *Milesia fructuosa* Faull, I on *Abies balsamea*, coll. Darker; *Pucciniastrum Arcticum* (Lagerh.) Tranz., II on *Rubus triflorus*, coll. Cornell; *Pucciniastrum Potentillae* Kom., II on *Potentilla tridentata*, coll. Cornell.

Fungi Imperfeci: *Cercospora Callae* (Peck) Clinton, coll. Cornell; *Cercospora gentianicola* Ellis & Ev., coll. Cornell; *Coccospora aurantiaca* Wallr., coll. Cornell; *Gelatinosporium fulvum* Peck, coll. Wehmeyer; *Fusicladium radiosum* (Lib.) Lindr., coll. Cornell; *Rhinotrichum repens* Preuss., coll. Linder; *Septoria Scutellariae* Thüm., coll. Cornell; *Septoria Wilsonii* Clinton, coll. Cornell; *Dendrodochium epistroma* v. Höhn. on *Diatrypella betulina* (Peck) Sacc., coll. Shear; *Macrophoma parca* (Berk. & Br.) Berl. & Vogl. on *Abies balsamea*, coll. Shear.

H. S. JACKSON

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#### ANNOUNCEMENT FOR 1935 SUMMER FORAY

The Council of the Society at the Atlantic City meeting delegated to the Vice-President the function of selecting the time and place for the summer foray. In accordance with this procedure Vice-President Dearness has announced that the 1935 foray will be held at Ithaca, New York, August 20-23 inclusive. The mycological laboratories of the Department of Plant Pathology in the Plant Science Building of Cornell University will serve as headquarters. The local committee in charge of arrangements will consist of Professor H. H. Whetzel and the Secretary-Treasurer. The Ithaca members of the Society extend a cordial invitation to all mycologists to attend.

Lodging and meals can be obtained at reasonable rates at rooming houses and restaurants on or bordering the campus. All who plan to attend the foray are asked to advise the Secretary-Treasurer well in advance in order that adequate arrangements may be made. It is especially urged that the type of accommodations desired be indicated. Also it is important that those who intend to arrive by train shall so state, in order that automobiles may be obtained for their use while at the foray. Camp sites are available near by for any who desire to use tents.

Ithaca is located in the scenic Finger Lakes region of central New York at the head of Cayuga Lake, and is well known to botanists for the natural beauty of its surroundings, and the richness and variety of its flora. Within easy reach are fresh water marshes and lakes, peat and marl bogs, numerous small gorges with many waterfalls, upland woods, and open fields. High hills afford a variation in elevation of more than fifteen hundred feet with a corresponding difference in flora. In most seasons fungi occur in profusion, and in only the most unusual August is the collecting unsatisfactory. Pleasant days and cool nights are to be expected. The laboratories are well equipped with facilities for handling the materials collected. The herbarium, containing the specimens of Atkinson and his students is available for consultation.

Members of the Society and other students of the fungi are urged to make careful note of the dates of the foray, and to arrange their summer plans to include it. The success of the meeting last summer at Seventh Lake indicates a growing interest in this phase of the Society's activities.—H. M. FITZPATRICK, Secretary-Treasurer.

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A committee named by the President at the Pittsburgh meeting submits the following resolution for publication in *Mycologia*:

During the year 1934, the Mycological Society of America has lost four of its members through death. The Society, therefore, expresses its deepest regret at their removal and extends its sympathy to the bereaved families. Those in whose memory this action is taken are: Charles E. Fairman; Mrs. Esther Lewis; Thomas H. Macbride; Frank Lincoln Stevens.

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#### TWO NEW SPECIES OF NEUROSPORA

In the course of the writer's interspecific hybridization studies in *Neurospora* on which a report will be published soon, it was found that an eight-spored strain of *Neurospora*, collected and isolated in 1927 at Nanking, China, is distinct morphologically and physiologically from the other two eight-spored species of *Neurospora* so far described. Morphologically it is intermediate

between them, having an ascospore of the *N. sitophila* type, and a perithecium and ascus of the *N. crassa* type. It was formerly thought to be simply a hybrid of *N. sitophila* and *N. crassa*. But recent physiological studies by the writer proved the incorrectness of his early assumption, as the Chinese strain of the sex-reaction "a" produces on dextrose agar an abundance of saffron-colored conidia, quite distinct from the salmon-pink colored conidia of *N. sitophila* and *N. crassa* on the same medium. This Chinese strain also hybridizes rather readily with all of the species of *Neurospora* so far available and it is the only species that has to date been crossed with the new species from Porto Rico. It is, therefore, deemed proper to describe the Chinese strain as a new species.

#### *Neurospora intermedia* sp. nov.

Peritheciis gregariis vel sparsis, atro-brunneis, 310-640  $\mu$  in diametro, ostiolo papillari; ascis cylindricis, brevissime stipitatis, 170-220  $\mu$   $\times$  15  $\mu$ , 8-sporis; sporidiis uniseriatis, ellipticis, viridi-atris, longitrussum striatis, 19-26  $\mu$   $\times$  12-15  $\mu$ , typice 23  $\mu$   $\times$  13  $\mu$ .

Conidia in mass saffron-colored on dextrose agar, cantenulate, globose to ovate, 11-21 by 10-11  $\mu$ .

Hab. on corn cob, Nanking, China. Type deposited at The New York Botanical Garden and also at the Institute of Agricultural Research, Tsing Hua University, Peiping, China.

A four-spored species of *Neurospora* was collected by Dr. R. A. Toro in Puerto Rico, and the cultures were kindly provided for study by Dr. B. O. Dodge. This species does not hybridize, at least readily, with *N. sitophila*, *N. crassa* and *N. tetrasperma* as was found by Dodge<sup>1</sup> and recently confirmed by the writer. It is, however, usually fertile with the Chinese species, *N. intermedia*, described above. Again the component unisexual strains of the Toro species are fluffy when grown on dextrose agar, producing quite an abundance of yellowish conidia, while bright orange-colored conidia are produced by the strain of sex-reaction "a" of *N. tetrasperma*.

Morphologically it also differs from *N. tetrasperma* by its larger perithecium and also longer and narrower asci. The

<sup>1</sup> Dodge, B. O. The non-sexual and the sexual functions of microconidia of *Neurospora*. Bull. Torrey Bot. Club 59: 347-360. 1932.

ascospores of the Toro species, although having the same range of size as those of *N. tetrasperma*, are as a rule longer and narrower. The ascus of *N. tetrasperma* according to the writer's measurement, is 128–195  $\mu$  in length and 19–24  $\mu$  in width, mostly 144 by 21  $\mu$ . Most of the ascospores are about 30  $\mu$  long and 16  $\mu$  wide. The size of the peritheciun, varying much, does not seem to be a good criterion for distinguishing species of *Neurospora*. It seems that when few perithecia are formed, they are larger. Thus, the peritheciun of *N. tetrasperma* usually has a diameter of 249  $\mu$  to 332  $\mu$ , but the diameter sometimes reaches 664  $\mu$  when few perithecia are produced.

Since the Toro strain is distinct morphologically and physiologically from the other four-spored forms of *Neurospora*, it is described as a new species.

#### *Neurospora Toroi* sp. nov.

Peritheciis gregariis vel sparsis, fuscis vel atris, 370–580  $\mu$  in diametro, ostio papillari; ascis cylindricis, stipitatis, 168–216  $\mu$   $\times$  18–21  $\mu$ , typice 192  $\times$  18  $\mu$ , 4-sporis, interdum 3 vel 5 sporis; sporidiis uniseriatis, oblongo-ellipticis, longitrus striatis, fuscis vel atris, 27–37  $\mu$   $\times$  14–18  $\mu$ , typice 32  $\times$  14  $\mu$ .<sup>1</sup>

Conidia pink-colored in mass on corn meal agar, yellowish on dextrose agar, conidia cantenulate, globose to ovate 8–16 by 8–11  $\mu$ .

Hab. in coffee soil in Puerto Rico. Type deposited at The New York Botanical Garden.

F. L. TAI

THE NEW YORK BOTANICAL GARDEN

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#### A MUCOR FOUND IN FOWL<sup>2</sup>

While conducting nutrition experiments with young chickens, there was evident, a lack of thrift and a mortality not attributable to the type of feeding. Some of the dead birds and others that were killed were examined anatomically. In all cases the gizzard was highly inflamed and the mucous membrane had become separated from the heavy epithelial layer. The infected material

<sup>1</sup> Only the measurements of the ascospores in four-spored asci are given here.

<sup>2</sup> Journal Series paper of the N. J. Agricultural Experiment Station, Department of Seed Analysis.

from between the mucous and muscular coats was cultured upon nutrient agar, resulting in a pure culture of a filamentous fungus from all birds so examined.

When grown on nutrient agar, there were found monilioid hyphae and aerial budding similar to that of the *Monilia sitophila* group; but differing from the *Monilia albicans* type in which the yeast-like growth is predominant. This suggested a monilioid phase of a *Mucor*. Transfers made to sterile bread produced luxuriant growth and sporulation of a *Mucor* which belongs near to *Mucor hiemalis*, Wehmer (1) and *Mucor javanicus* Wehmer (2) and is probably a transitional form between the sub-genera, *Mono-* and *Cymo-Mucor* having both simple and sympodially branched sporangiophores. The monilioid phase of these two organisms has been cited by Lendner (3) as the Chinese yeast in oriental fermentation processes.

Whether or not this organism is the primary cause of the pathological condition or only a secondary factor is uncertain, for time and space to conduct confirmatory experiments were not available at the time. For this reason the information is reported as an observation.

This organism so obtained from the gizzards of chicks is added to Dr. Lockwood's collection of Mucorales under the name of *Mucor javanicus* 461

1. Wehmer, C. Der *Mucor* der Hanfroette, *M. hiemalis*, nov. spec. Ann. Myc. 1: 37-41. 1903.
2. —. Der javanische Ragi und seine Pilze, Zentralb. Bakt. Abt. II, 6: 610-619. 1900.
3. Lendner, A. Les Mucorinees de la Suisse, Berne, 180 pages. 1908.

NANDOR PORGES, JULIUS F. MULLER, AND LEWIS B. LOCKWOOD

NEW JERSEY AGR. EXP. STATION,  
U. S. DEP. OF AGRIC.

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#### THE LICHEN FLORA OF THE UNITED STATES

The writer is especially interested in seeing this volume since he was closely associated with Dr. Fink, both in Iowa and since coming to The New York Botanical Garden. Miss Joyce Hedrick (Mrs. Volney H. Jones) is to be congratulated on her persistence in completing the work cut short by Dr. Fink's death.

As is well known, Dr. Fink believed that the lichens or algicolous fungi should be combined with parallel groups of non-algicolous fungi. The present volume has not gone as far in that direction as had been anticipated.

We note that the order Hysteriales is used as a lichen order, but includes only families of algicolous fungi, while Dr. Fink in previous publications included also the non-algicolous families, Hysteriaceae and Hypodermataceae. While this has not been done in the present volume, we assume that it was intended, otherwise the author would scarcely have been justified in using an ordinal name previously applied only to non-algicolous fungi. Undoubtedly other families of non-algicolous fungi will eventually be included also in the Lecanorales. However, the problem of deciding how non-algicolous fungi and the algicolous forms should be interspersed is a large one and Miss Hedrick apparently preferred not to deal with that problem here.

As indicated, most of the lichens of North America are ascolichenes, and it is a great convenience to have the species brought together under one cover. The volume consists of 426 pages of text, including an index of 33 pages, with all the species arranged alphabetically and the genus in parenthesis following, thus making it easy to locate any given species in the least possible time. The volume is illustrated with 47 plates consisting mostly of half-tones with a few etchings. There are also a number of text figures taken from Schneider to illustrate the morphological characters of the lichen.

The introductory matter has been partly reprinted from the "Lichens of Minnesota," but modified somewhat to suit Fink's later views. One hundred and seventy-eight genera are treated in this volume. The specific diagnoses are brief and concise, an effort having been made to avoid repetition of generic characters. While the writer is not thoroughly familiar with lichen fungi, the book appears to be a very concise and thorough treatment of this group for the United States.

The volume is published by the University of Michigan Press, Ann Arbor, Michigan (price \$4.00).—FRED J. SEAVER





*PLRUROTUS CORTICATUS*

# MYCOLOGIA

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## THE PRODUCTION OF ASEXUAL SPORES BY PLEUROTUS CORTICATUS<sup>1</sup>

FRANK KAUFERT<sup>2</sup>

(WITH PLATES 26-31)

Asexually produced spores of several types, conidia, oidia, and chlamydospores, have been described for many species of the Agaricales, but they and the structures upon which they are formed are usually inconspicuous. Such spores have been described as being formed by segmentation of single hypha, or upon the tips of specialized branches of individual hypha. They are usually formed only in culture and can be found only by close microscopic examination of the vegetative mycelium. Compound fruiting bodies, such as coremia, accervuli, and pycnidia, apparently have not been described for the Agaricales.

While making a study of fire and decay damage in the bottomland hardwood region of northeastern Louisiana in 1931, the writer isolated an agaric which formed tall, white coremia, capped with glistening masses of black conidia. The fungus was isolated from the incipient decay in a red gum tree (*Liquidambar styraciflua*) which had been fire-scarred 15 years previously. The

<sup>1</sup> Paper No. 1295 of the Journal Series of the Minnesota Agricultural Experiment Station.

<sup>2</sup> The writer wishes to express his indebtedness to Dr. E. C. Stakman, under whose direction this work was done, and to Dr. L. O. Overholts, Department of Botany, Pennsylvania State College, for identifying several sporophores sent him.

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production in culture of these striking compound sporophores and asexual spores by a wood-destroying Basidiomycete stimulated study of the fungus, and especially of the accessory spores.

Fortunately, small sporophores were formed in culture when the fungus was grown on 4.0 per cent malt extract agar. The production of these small, white, generally imperfect sporophores with eccentric stipes and decurrent gills suggested that the fungus was possibly a *Panus* or a *Pleurotus*. The following spring a *Pleurotus*-like sporophore was collected near Greenville, Mississippi, and, while it was drying, white coremia and black conidial heads developed on the gills, pileus, and stipe. Tissue cultures made from this sporophore also produced an abundance of coremia and black conidial heads similar to those formed upon the sporophore and those found in the culture made from the decayed wood the previous season.

This sporophore, which unfortunately was in poor condition, was sent to Dr. L. O. Overholts. On the basis of the unusual elongate-cylindrical basidiospores, 12.5–19 by 4.3–6.0  $\mu$ , and other recognizable characteristics, he tentatively identified it as *Pleurotus corticatus*. In the past year the fungus has been grown on a number of media that induce fruiting of many stipitate agarics, and several fairly large sporophores have formed. Although they were no doubt abnormal in some respects, they resembled closely the original sporophore. From the original sporophore and those formed in culture the following description may be given for the fungus.

Pileus compact, firm when dry, convex to expanded, 4–8 cm. broad, dull white at first, becoming grey to yellow-brown with age, finely floccose at first, becoming almost squamose toward the stipe at maturity, solitary or occasionally cespitose. Flesh thick, white, brittle. Gills white, becoming yellow with age, decurrent, occasionally anastomosing toward the stipe, edge entire, rather broad in front, tapering toward the base. Stem 4–8 cm. long, lateral in most specimens, solid, slightly pubescent, tapering considerably. Veil rarely formed on sporophores developed in culture, fugacious, rarely leaving an annulus; when present it consists of small scale-like remnants closely appressed to the stipe. There is no evidence of a veil on the original sporophore. Basidiospores cylindric-elongate, 12.5–19 by 4.3–6.0  $\mu$ , smooth, pure-white in mass. Sporophores becoming covered

with tall white coremia, bearing glistening black drops of conidia at their tips, when placed in a humid chamber or when dried slowly.

This description agrees closely with the one given for *Armillaria corticata* Fries-Pat., by Kauffman (4). Kauffman discarded the old subdivision of the genus *Pleurotus*, set up by Fries to include the two species possessing a fugacious veil, *Pleurotus corticatus* and *Pleurotus dryinus*, and has transferred these species to the genus *Armillaria*. The only really significant difference between the two species is in spore size, those of *Armillaria (Pleurotus) corticata* being larger than those of *Armillaria (Pleurotus) dryina*, which are 9–10 by 4–4.5  $\mu$ . Atkinson (1) considers *Pleurotus corticatus* merely a form of *P. dryinus*. Murrill's description (5) for *Pleurotus dimidiatus* (Schaeff.) Murrill also fits my fungus quite well, the spore dimensions he gives being somewhat smaller, corresponding with those given for *Armillaria dryina* by Kauffman.

#### SPOROPHORE PRODUCTION

The fungus grows well upon malt extract agar, producing a luxuriant mycelium, an abundance of coremia, aborted sporophores, and occasionally a sporophore with gills and pileus. But nutrient agar, even in large quantities, does not induce the degree of vegetative development prerequisite to sporophore production by the larger stipitate agarics. To obtain sufficient vegetative growth it is necessary to have a medium that is porous and contains an abundance of food material, as pointed out by Etter (3). Such a medium was obtained by adding a 4.0 per cent malt extract solution to basswood sawdust in quart jars. Six weeks to two months after inoculation the mycelium usually filled the jar and had begun to form sporophore primordia. The quart jars were then placed under bell jars and the caps removed. Abortive sporophores, such as those shown in plate 26, figures 1 and 2, were commonly formed, but when the conditions were exactly right, large, apparently normal sporophores, such as the one shown in plate 27, figure 1 and 2, were formed. Such sporophores produced an abundance of basidiospores. From the numerous tests that have been made upon many media under a large variety of condi-

tions, the requirements for sporophore production by *Pleurotus corticatus* appear to be the following: A porous medium containing an abundance of sugars, particularly maltose; rather high temperatures, 27 degrees C., being the optimum for vegetative growth. Abundant vegetative growth is a necessary preliminary to sporophore production. Once the necessary vegetative growth has been made at the high temperature, sporophore production will take place at a lower temperature. Sunlight or diffuse light is necessary for sporophore production, although vegetative growth is most rapid in the dark.

#### BASIDIOSPORES

The basidia of the fungus are four-spored. The basidiospores are 12.5–19.0  $\mu$  by 4.3–6.0  $\mu$  and contain a single nucleus (PLATE 30, FIG. 1). On germination a haploid mycelium is produced. This differs from the binucleate mycelium in rate of growth and in the absence of clamp connections, but, like the dicaryotic mycelium, it forms coremia and conidia.

It has been very difficult to induce the basidiospores to germinate. Although a large number of media and stimulatory substances have been tried under many different conditions, it has been possible to obtain but five haploid lines. The conditions required for germination have not yet been determined.

From the results of mating experiments with the five haploid lines and diploidization tests with the parent dicaryotic mycelium and these haploid lines, it is evident that the fungus is heterothallic. Further work upon the sexuality and nuclear phenomena of the fungus is now under way and will be reported on later.

#### CONIDIA

In addition to the conidia which are formed in the tall coremia on both the binucleate and haploid mycelia, simple conidia also are produced, single spores being formed on short conidiophores. Therefore, four types of conidia are formed: binucleate conidia formed in coremia on the dicaryotic mycelium; simple, binucleate conidia borne singly at the tips of short hyphal branches on the dicaryotic mycelium; uninucleate conidia formed in coremia on the haploid mycelium; and simple, uninucleate conidia borne singly at the tips of short conidiophores on the haploid mycelium.

*Binucleate conidia in coremia.* Binucleate conidia are formed in coremia on perfect sporophores, abortive or abnormal sporophores, and upon the dicaryotic mycelium when the fungus is grown on an agar medium.

When sporophores are placed in a humid chamber or are dried slowly, they soon become covered with tall white coremia, each capped by a black glistening drop of conidia (PLATE 27, FIG. 3 AND 4, AND PLATE 28). The coremia begin as small tufts of white mycelium upon the stipe and gills, these increasing in diameter and height until in some cases they may be 2 cms. tall and 0.5 cm. in diameter. The coremia formed upon the gills are normally much smaller than those on the stipe. After 4 days in a damp chamber spore formation commences and the coremial heads become black and glistening. After two weeks in a humid chamber the sporophores have shrunken somewhat, as seen in plate 27, figure 4, and are covered with coremia. The sporophores show a remarkable resistance to decay by molds and bacteria, for even after 2 weeks in such a chamber the sporophores are not molded or decomposed badly.

Coremia and conidia of the same type as are formed upon normal sporophores are formed in culture on abnormal or aborted sporophores, which are produced abundantly on any medium that permits vegetative growth. Plate 26, figure 1, and plate 29, figure 1, illustrate this type of sporophore formation. The aborted sporophores are often completely covered and blackened by such coremia (PLATE 29, FIG. 2).

Coremia are also formed directly on the binucleate vegetative mycelium when the fungus is grown upon nutrient media. The coremia have essentially the same structure and are of about the same size as those formed upon normal and abnormal sporophores (PLATE 30, FIG. 1 AND 2).

The coremia formed on the binucleate mycelium are composed of tightly compacted masses of hyphae which have prominent clamp connections at every cross wall. At the tips of the coremia the mycelium is less tightly compacted, the free ends of the hyphae breaking up in a basipetal manner into chains of conidia. The structure of the coremia and method of spore formation are illustrated in plate 31, figures 4 and 6. The clamp connection

becomes a part of the spore formed beneath the septum on which the clamp occurs. The terminal spores of a chain are usually rounded and mature. Below these are others with distinct beak-like projections, the remnants of the old clamp connections. Deeper in the head are still more immature spores with clamp connections still attached and distinct. The conidia vary greatly in size, the spores in a single head sometimes ranging from 9.0 to 27.0  $\mu$  in length and from 4.0 to 8.5  $\mu$  in width. The walls of the spores are yellow-brown, appearing black in masses and when wet. The liquid in which they are formed is colorless, the drop at the tip of the coremia obtaining its black color from the mass of dark-walled spores. The nuclei of the conidia are easily stained with iron-alum-haematoxylin, there usually being two nuclei in each spore. A few spores have been found, however, which contained but a single nucleus. Whether or not these are actually haploid conidia formed upon the dicaryotic mycelium, as described for *Pholiota aurivella* by Martens and Vandendries (6), is still uncertain. All the conidia that have been germinated have given rise to binucleate mycelia with prominent clamp connections at every cross wall. The number of conidia formed in a single head is astonishing. The spores from a single head were washed onto a slide, evenly distributed, and counts made on small sample areas. On the basis of such counts a single head 1.0 mm. in diameter was found to contain about 10,000 spores. Some of the larger heads, which may be 7 mm. in diameter, probably contain over a million spores.

*Simple binucleate conidia.* In addition to the binucleate conidia in coremia, the fungus forms a second type of binucleate conidia, single binucleate spores borne singly at the tips of small conidiophores (PLATE 31, FIG. 2). These spores appear to be formed only when the fungus is growing on a medium poor in nutrients or when the fungus is grown under adverse temperature and moisture conditions. The spores are small, round, and vary from 2.5 to 4.2  $\mu$  in diameter.

*Uninucleate conidia in coremia.* The haploid mycelium of *Pleurotus corticatus*, resulting from a single basidiospore, differs from the dicaryotic mycelium in rate of growth, the growth of the haploid lines being about one-half as fast as that of the dicaryotic

mycelium; in the absence of clamp connections; and in the lack of sporophore formation. The haploid mycelium resembles the dicaryotic in that it forms conidia in coremia and also simple conidia. The coremia formed on the haploid mycelium are usually smaller than those on the dicaryotic mycelium but have the same general structure (PLATES 30 AND 31). The structure of the haploid coremia is shown in plate 31, figure 7, and the method of spore formation in figure 5. The uninucleate conidia vary greatly in size; the spores from a single head may vary from 4.5 to 15.0  $\mu$  in length and from 4.0 to 7.0  $\mu$  in width. The uninucleate conidia are thus considerably smaller than the binucleate conidia. Upon germination these uninucleate conidia always give rise to mycelia of the same sex as those upon which they were produced. Although the conidial heads formed on the haploid mycelium are smaller than those formed on the dicaryotic mycelium, the spores produced are equally numerous because they are much smaller. The uninucleate conidia are produced in basipetal succession, have yellow-brown walls, and are borne in a drop of a thin colorless liquid. From the appearance of these spores and the sticky fluid in which they are formed it would seem that they probably are disseminated by insects in nature, much like the haploid oidia of *Coprinus Lagopus* described by Brodie (2).

*Simple uninucleate conidia.* Like the dicaryotic mycelium the haploid mycelium forms simple conidia, borne singly upon short conidiophores, when grown under adverse conditions. These spores are always uninucleate, are small, 1.7–3.5  $\mu$ , and have yellow-brown walls. This difference in size of the simple binucleate conidia and the simple uninucleate conidia constitutes another constant difference between the haploid and dicaryotic mycelia. The way these spores are borne on the haploid mycelium is shown in plate 31, figure 3.

#### SUMMARY

1. The production of asexual spores by *Pleurotus corticatus* Fries is described.
2. The formation of conidia on coremia is described for an agaric for the first time, as far as the writer is aware.

3. Binucleate conidia are formed in coremia on normal sporophores, if the sporophores are placed in a humid chamber while still fresh; upon abnormal or aborted sporophores; and upon the dicaryotic vegetative mycelium when the fungus is grown upon a nutrient medium.

4. Simple binucleate conidia are formed singly at the tips of small conidiophores on the dicaryotic mycelium when the fungus is grown under adverse conditions.

5. Uninucleate conidia are formed on coremia on the haploid mycelia derived from single basidiospores.

6. Simple uninucleate conidia are formed singly at the tips of small conidiophores on the haploid mycelium when the fungus is grown under adverse conditions.

7. Fairly large sporophores of the fungus may be produced on a medium composed of basswood sawdust and malt extract. Basidiospores from such sporophores do not germinate very readily.

8. From tests with the few haploid lines obtained it has been established that the fungus is heterothallic.

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ST. PAUL, MINNESOTA

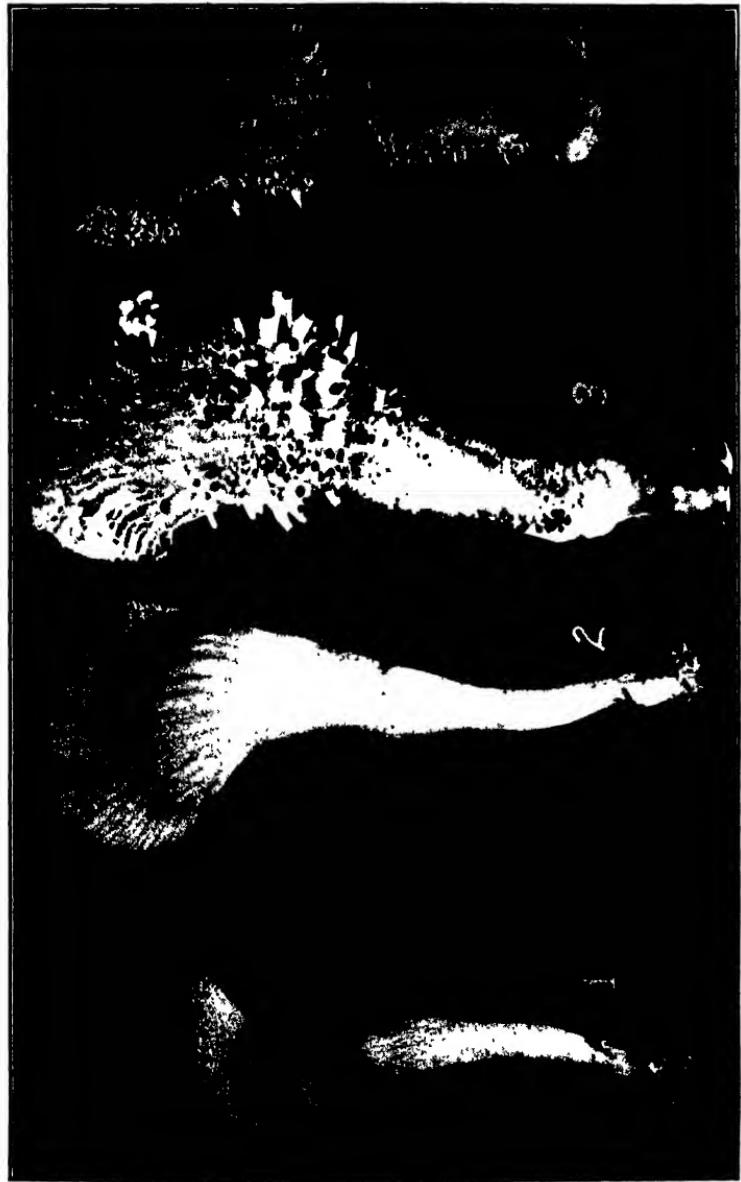
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#### EXPLANATION OF PLATES

##### PLATE 26

Fig. 1, abortive primary and secondary sporophores of *Pleurotus corticatus* produced on a mixture of basswood sawdust and malt extract but under light and humidity conditions that were unfavorable for the fungus; 2, abnormal sporophores of *Pleurotus corticatus* formed upon a mixture of basswood sawdust and malt extract when subjected to too intense light which causes the entire pileus to become inverted.

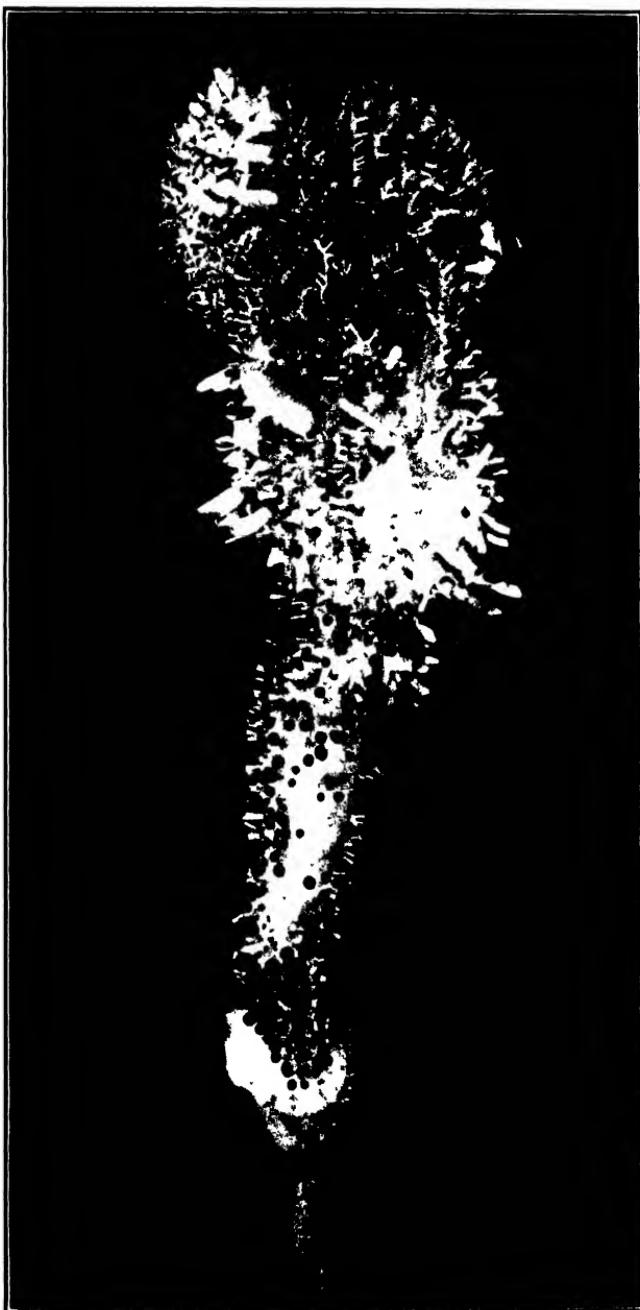


*PLEUROTUS CORTICATUS*



MYCOLOGIA

VOLUME 27, PLATE 28



PLEUROTUS CORTICATUS





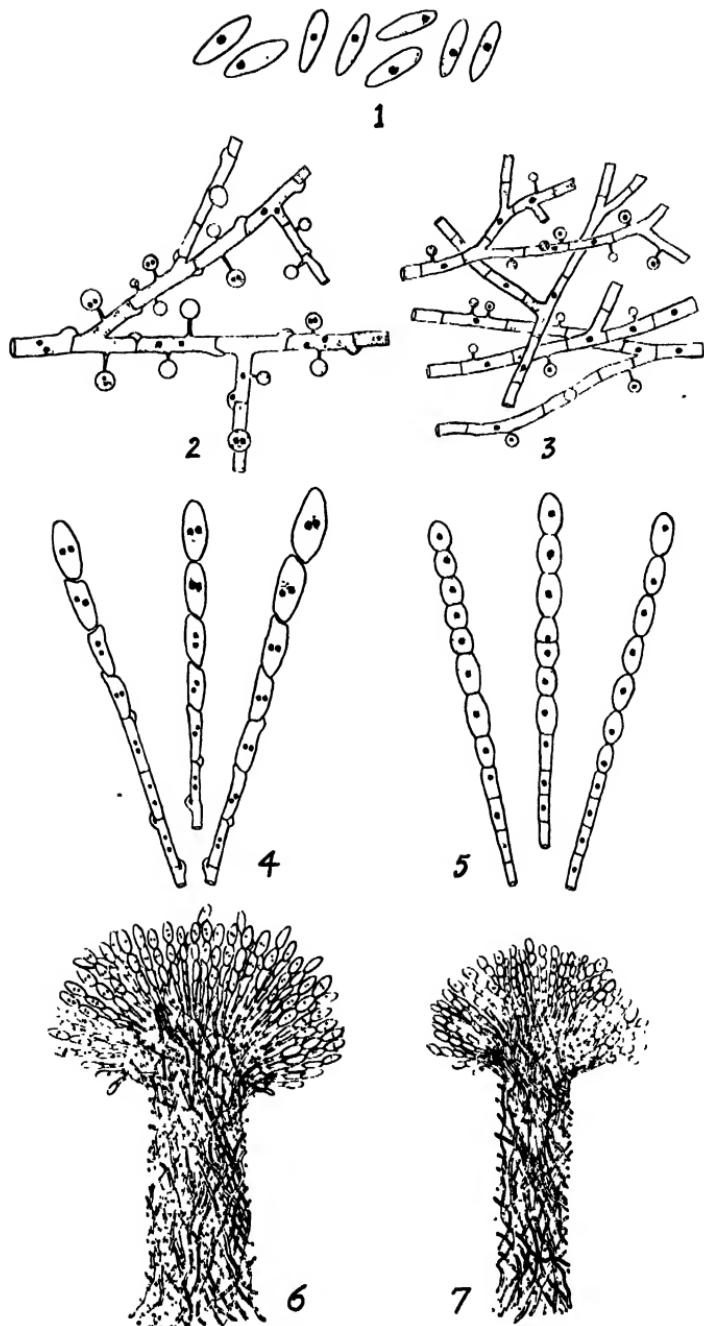
PLEUROTUS CORTICATUS





**PLEUROTUS CORTICATUS**





PLEUROTUS CORTICATUS



## PLATE 27

A sporophore of *Pleurotus corticatus* formed in pure culture upon a mixture of basswood sawdust and 5 per cent malt extract. Natural size. Fig. 1, top view of the sporophore showing the long eccentric stipe and the dark color of the pileus; 2, lower surface of the sporophore showing the decurrent gills; 3, the same sporophore after 6 days in a chamber in which the humidity was kept high, tall white coremia with glistening black drops of binucleate conidia at their tips having developed on the stipe and gills; 4, the same sporophore after 12 days in the humid chamber, shrunken in size and many more coremia and conidial heads having developed upon the stipe and gills.

## PLATE 28

The same sporophore as is shown in plate 27, figure 4, but enlarged to about  $1\frac{1}{2}$  times natural size to show the formation of coremia and conidia upon the gills and stipe in greater detail. Some of the abortive secondary sporophores shown in plate 27, figure 3, were broken off when the photo was made.

## PLATE 29

Fig. 1, abortive sporophores and coremia upon a mixture of malt extract and basswood sawdust, the abortive sporophores developing first and then covered by the tall white coremia and black conidial heads (a humid atmosphere in the container is necessary for the formation of coremia upon such aborted sporophores), natural size; 2, a single aborted sporophore enlarged about 3 times to show the nature of the coremia and black spore heads which have formed on them. (Note the secondary aborted sporophores mixed with the coremia.)

## PLATE 30

*Pleurotus corticatus* growing upon malt extract agar. Fig. 1, the large colony at the left is dicaryotic, the smaller one at the right haploid, resulting from a single basidiospore (note the difference in rate of growth of haploid and dicaryotic cultures, the dicaryotic mycelium normally growing almost twice as fast as the haploid, also the difference in size of coremial heads upon the dicaryotic and haploid mycelium), natural size; 2, a small section of the dicaryotic culture is shown at the left and a section of the haploid mycelium at the right, both having been enlarged about 3 times, the difference in size of the dicaryotic and haploid coremia brought out very clearly here.

## PLATE 31

The spores of *Pleurotus corticatus*. All drawings made with the aid of a camera lucida and the preparations stained with iron-alum haematoxylin. Figures 1-5 approximately  $\times 500$ . Fig. 1, basidiospores of the fungus obtained from the sporophore shown in plate 27; 2, binucleate conidia formed singly upon the dicaryotic mycelium when it is grown upon malt extract agar; 3, uninucleate conidia formed singly upon the haploid mycelium grown from a single basidiospore; 4, binucleate conidia or oidia from a coremium formed upon the dicaryotic mycelium (note that the clamp connections gradually disappear as the spores mature, becoming a part of spore beneath the septum); 5, uninucleate conidia or oidia from a coremium formed upon the haploid mycelium (note the extreme variation in size and shape of these spores); 6, section through a coremium formed upon the dicaryotic mycelium, showing the structure of the coremia and the way the binucleate conidia are borne in the heads, about  $\times 200$ ; 7, section through a coremium formed upon the haploid mycelium, about  $\times 200$ .

# PESTALOTIA SPP. ON AUCUBA, CIBOTIUM AND LEUCOTHOË

R. P. WHITE

(WITH PLATE 32)

During the course of investigations upon the pathogenicity of various species of *Pestalotia*, three undescribed species have come to attention. The species on *Aucuba japonica* var. *variegata*, and *Leucothoë Catesbaei* are weak wound parasites. The type on *Aucuba* follows the large black leaf spots caused by *Colletotrichum Pollacci* Magn. and occurs generally on sun scalded areas. The type on *Leucothoë* follows infections of *Cryptostictus* sp. and *Lophodermium* sp. as well as injuries to the foliage caused by drying during the winter. The species on *Cibotium Schiedei* has been proved a primary pathogene, causing the destruction of frondlets and entire fronds under the conditions of commercial culture as practiced for this fern in greenhouses.

These three species of *Pestalotia* have been examined critically both on their respective hosts and in culture on a variety of standard media.<sup>1</sup> All three forms are considered new species, since they occur on hosts on which species of *Pestalotia* have not previously been described, and are unlike species which occur on related hosts of the same family.

## *Pestalotia Aucubae* sp. nov.

Conidia 5 cellularia fusoides 23.6–31.5 × 6.9–8.8  $\mu$  (plerumque 25.5–29.5 × 6.9–7.8  $\mu$ ); duae superiores coloratae cellulæ obscuriores inferiore olivacea cellula. Superior cellula hyalina turbinata, setae plerumque 3, generaliter erumpentes ex eodem punto in apice cellulæ superioris, late divergentes, 21–51  $\mu$  longae, plerumque 29–41  $\mu$ . Conidiophoris filiformibus plerumque 7.8–9.8  $\mu$  longis. Acervuli 150–300  $\mu$  in diam., generaliter epiphilli.

Conidia 5-celled, fusiform, erect or slightly curved, only slightly constricted at septae, 23.6–31.5  $\mu$  long (usually 25.5–29.5  $\mu$ ; ave. 28.1  $\mu$ ) by 6.9–8.8  $\mu$  wide (usually 6.9–7.8  $\mu$ ; ave. 7.7  $\mu$ ); colored cells 13.8–19.7 (usually 15.7–17.7  $\mu$ ; ave. 16.9  $\mu$ ); the walls and

<sup>1</sup> Thanks are due to Dr. E. F. Guba for critical examinations of the species as well as for suggestions pertaining to their description.

septae dark, the upper two colored cells darker than lower colored cell, becoming umber or darker with age, guttulate; wall between two upper colored cells usually very dark; apical hyaline cell conic, setae commonly 3, rarely 2, 4 or 5, united or arising at the apex of superior cell, rarely arising from different points, widely divergent, slender,  $21.7\text{--}51.22\ \mu$  long (generally  $29\text{--}41\ \mu$ ; ave.  $37.6\ \mu$ ); basal cell typically long conic, pedicel filiform  $2.0\text{--}17.7\ \mu$  long (usually  $7.8\text{--}9.8\ \mu$ ; ave.  $9.5\ \mu$ ), generally erect. Pustules  $150\text{--}300\ \mu$  in diameter usually epiphyllous, scattered, the contents issuing as coils surrounded by the torn epidermis or as compact cones.

On foliage of *Aucuba japonica* var. *variegata* following infections of *Colletotrichum Pollaccii* Magn. (PLATE 32 A) or injuries due to sun scald. A secondary pathogene. Type locality, Rutherford, N. J. August 20, 1932.

In culture heavy white aerial growth is produced on potato dextrose and Sorbaud's potato dextrose, with scant aerial growth on lima bean, bean pod and corn meal agars and none on prune agar. Sporulation is abundant on potato dextrose, lima bean and bean pod agars in both petri dishes and tubes, but scant on Sorbaud's potato dextrose, corn meal and prune agars. When grown on potato dextrose agar the medium becomes "light ochraceous buff"<sup>2</sup> to "ochraceous buff"; on Sorbaud's potato dextrose agar the medium becomes "raw sienna" when viewed from below.

### Pestalotia Leucothoës sp. nov.

Conidia 5-cellularia, constricta fusoideia, infera acuta  $21.5\text{--}29.5\ \mu \times 4.9\text{--}6.9\ \mu$  (plerumque  $23.5\text{--}27.5 \times 5.5\text{--}6.3\ \mu$ ); superiores et mediae cellulae coloratae obscuriores quam inferior olivacea cellula. Superior cellula hyalina longa turbinata, setae 2-5 plerumque 3 vel 4 saepe erumpentes ex aliis punctis in corona cellulae superioris, raro ramosa similis cervi cornu, usque ad  $39.5\ \mu$  longae, plerumque  $20\text{--}33\ \mu$ . Conidiophoris plerumque  $6\text{--}14\ \mu$  longis, usque ad  $16\ \mu$ . Acervulis  $75\text{--}150\ \mu$  in diam., subglobosis, plerumque hypophyllis.

Conidia 5-celled, narrow fusiform, acute at base, erect or usually curved, only slightly constricted at septae  $21.5\text{--}29.5\ \mu$  long (usually  $23.5\text{--}27.5\ \mu$ ; ave.  $25.9\ \mu$ ) by  $4.9\text{--}6.9\ \mu$  wide (usually  $5.5\text{--}6.3\ \mu$ ; ave.  $6.2\ \mu$ ); upper and middle colored cells darker than the lowest olivaceous cell, guttulate; colored cells  $13.8\text{--}18.7\ \mu$  (usually  $15.7\text{--}17.7\ \mu$ ; ave.  $16.0\ \mu$ ). Apical hyaline cell long conic,

<sup>2</sup> Ridgeway's Color Standards.

setae 3 or 4, typically 3, rarely 2 or 5, often arising from different points on the crown of the apical cell; one or two setae sometimes arising below the summit, rarely branched like a staghorn,  $3.9\text{--}39.5\ \mu$  long (usually  $20\text{--}33\ \mu$ ; ave.  $26.1\ \mu$ ); basal cell, long acute tapering to pedicel; pedicel  $0\text{--}15.7\ \mu$  long (usually  $5.9\text{--}13.8\ \mu$ ; ave.  $9.6\ \mu$ ). Pustules  $75\text{--}150\ \mu$  diam., subglobose, largely hypophyllous, the black contents issuing in coils surrounded by torn epidermis, or oozing forth as a globose head, staining the matrix.

On foliage of *Leucothoë Catesbaei* following infections of other fungi and winter injury (PLATE 32 B AND C). A secondary pathogen. Type locality, Springfield, N. J. August, 1931.

On potato dextrose and Sorbaud's potato dextrose agars, a heavy white aerial growth of mycelium is produced with abundant sporulation appearing after 6 days in tubes and 12 days on plates. Sporulation is sparse on lima bean and bean pod agars and the vegetative growth is largely embedded in the media. On corn meal and prune agar, growth is entirely submerged in the media and sporulation is either limited to the point of inoculation or absent entirely in petri dishes. When grown on potato dextrose agar, the medium becomes "pale yellow-orange"; on Sorbaud's potato dextrose "tawny olive."

#### **Pestalotia Cibotii** sp. nov.

Conidia 5 cellularia, anguste fusoideia,  $19.7\text{--}29.5 \times 4.9\text{--}6.8\ \mu$  (plerumque  $21.5\text{--}27.5 \times 4.9\text{--}5.9\ \mu$ ). Mediae coloratae cellulæ olivaceæ, duæ superiores paulo obscuriores inferioribus. Superior cellula hyaline turbinata, globosa in apice, setae 3 vel 4, raro 2 vel 5, erumpentes ex aliis punctis in globosa apice superioris cellulæ,  $5.9\text{--}27.6\ \mu$  longæ (plerumque  $15.7\text{--}19.7\ \mu$ ). Conidiophoris brevibus,  $1.9\text{--}5.9\ \mu$  longis. Acervuli  $120\text{--}175\ \mu$  in diam., plerumque epiphilli.

Conidia 5-celled, slender fusiform, usually erect or slightly curved, very slightly constricted at septae,  $19.7\text{--}29.5\ \mu$  long (usually  $21.5\text{--}27.5\ \mu$ ; ave.  $24.7\ \mu$ ) by  $4.9\text{--}6.8\ \mu$  wide (usually  $4.9\text{--}5.9\ \mu$ ; ave.  $5.7\ \mu$ ); median colored cells  $13.8\text{--}17.7\ \mu$  (mostly  $15.7\text{--}17.7\ \mu$ ; ave.  $16.0\ \mu$ ), olivaceous, upper two only very slightly darker than the lower, sometimes umber. Apical hyaline cell, conic rounded at the summit; basal cell, broad acute. Setae 3 or 4, typically 4, rarely 2 and 5, arising from different points on the rounded top of the apical cell,  $5.9\text{--}27.6\ \mu$  long (usually  $15.7\text{--}19.7\ \mu$ ; ave.  $17.0\ \mu$ ). Pedicel short, straight,  $1.9\text{--}5.9\ \mu$  long (usually  $1.9\text{--}4.9\ \mu$ ; ave.  $3.9\ \mu$ ). Pustules largely epiphyllous,

the black contents issuing in coils or as cones, subglobose, 120–175  $\mu$  diameter.

Parasitic on living fronds of *Cibotium Schiedei* under green-house conditions (PLATE 32 D). Type locality, Rutherford, N. J. August 20, 1932.

In culture, thick closely woven mats of aerial mycelium are formed on potato dextrose and Sorbaud's potato dextrose agars which with age become tinged with "naples yellow." Sporulation is fairly abundant on both media; in plates the acervuli are all embedded in the medium, but in tubes spores are produced abundantly in wet black glistening drops. Sporulation also occurs abundantly in tubes of lima bean agar, and less abundantly in tubes of corn meal and prune agars. It does not sporulate in plates of bean pod agar.

Physiologically these organisms can be distinguished by the presence or absence of sporulation on various media. *P. Aucubae* sporulates abundantly on potato dextrose, lima bean and bean pod agars but sparsely on Sorbaud's potato dextrose agar. *P. Leucothoës* either fails to sporulate on lima bean and bean pod agars in plates or does so only at the point of inoculation, and sporulates abundantly on Sorbaud's potato dextrose agar. *P. Cibotii* fails to sporulate at all on plates of bean pod agar, and produces numerous embedded acervuli on plates of Sorbaud's potato dextrose agar and lima bean agar. Their habits of growth on lima bean agar are quite distinct (PLATE 32, H, I, J).

Morphologically *P. Aucubae* may be distinguished by its wider spores, and by its long, widely divergent setae, preponderantly 3 per spore; *P. Leucothoës* by its shorter setae often arising at different points on the crown of the apical cell, typically 3 per spore but with approximately 30 per cent of the spores with 4 setae; *P. Cibotii* by its short setae, typically 4 per spore but with approximately 40 per cent with 3 setae, often arising from different points on the apical cell, and its extremely short pedicel.

Type specimens of these fungi have been deposited in the herbarium of the Department of Plant Pathology, Cornell University, Ithaca, New York and in the cryptogamic herbarium at Harvard University, Cambridge, Massachusetts.

## EXPLANATION OF PLATE 32

*A*, leaf spot of *Aucuba japonica* var. *variegata* caused by *Colletotrichum Pollaccii* Magn. secondarily invaded by *Pestalotia Aucubae*; *B*, *C*, leaves of *Leucothoë Catesbaei* showing winter injury and secondarily invaded by *Pestalotia Leucothoës*; *D*, frond of *Cibotium Schiedei* infected with *Pestalotia Cibotii*; *E*, spores of *Pestalotia Cibotii*  $\times 440$ ; *F*, spores of *Pestalotia Aucubae*  $\times 400$ ; *G*, spores of *Pestalotia Leucothoës*  $\times 400$ ; *H*, *I*, *J*, cultures of *P. Aucubae*, *P. Leucothoës* and *P. Cibotii* respectively, on lima bean agar; 10 days old.



PESTALOTIA SP.



# THE PERFECT STAGE OF CERCOSPORA RUBI

FREDERICK A. WOLF<sup>1</sup>

(WITH 8 TEXT FIGURES)

## INTRODUCTION

The writer undertook a study of *Cercospora Rubi* Sacc., the cause of a common leafspot disease of *Rubus*, because so little is known regarding this pathogen. As a result of this investigation it was found that this conidial stage, present upon the leaves during summer, is succeeded by a perithecial stage which is initiated during autumn, but does not mature until early in the following spring. The following account of this study is herewith presented, therefore, as a contribution to our knowledge of the morphology and development of this leafspot fungus on *Rubus*.

Apparently both wild and cultivated species of *Rubus*, including blackberries, dewberries, and raspberries, are subject to attack by this imperfect fungus. Moreover, it is of common occurrence and widespread distribution within the eastern United States, and, in certain years, it occasions severe premature defoliation, especially in commercial plantings. Undoubtedly, a decrease in yield proportional to the degree that such defoliation interferes with the storage of food in the turions, results from this leafspot disease.

In North Carolina, the fungus has been collected on *Rubus*

<sup>1</sup> Contribution no. 136 from the Cryptogamic Laboratories of Harvard University. An investigation conducted while the writer held a Research Fellowship at Harvard University. Acknowledgment is made of the courtesies extended, for the loan of type specimens of species of *Sphaerella* on *Rubus*, by Dr. Gustav von Moesz, Director, Hungarian National Museum, Budapest, Hungary, by Dr. G. Samuelsson, Naturhistoriska Riksmusset, Stockholm, Sweden, and by Dr. J. A. Stevenson, Bureau of Plant Industry, Washington, D. C. The writer is grateful to Dr. Charles Chupp, Cornell University, Ithaca, N. Y., who confirmed the identification of the conidial stage as *Cercospora Rubi* Sacc. and gave his opinion on synonymy. Lastly, he is appreciative of the encouragement and assistance given by Messrs. W. H. Weston, Jr., and D. H. Linder.

*alleghaneensis* Porter, *R. argutus* Link, *R. hispidus* L., *R. procumbens* Muhl., *R. procumbens* var. *roribaccus* Bailey, *R. trivialis* Michx., *R. idaeus* L., *R. strigosus* Michx., *R. occidentalis* L., and *R. thyrsoides* Wimm. Among other species known to be suspects are *R. fruticosus* L. and *R. imperialis* Cham. It appears probable that an exhaustive examination of collections in herbaria, to include both those in this country and abroad, would reveal that *Cercospora Rubi* is pathogenic to essentially all of the numerous species of *Rubus*.

#### CONIDIAL STAGE

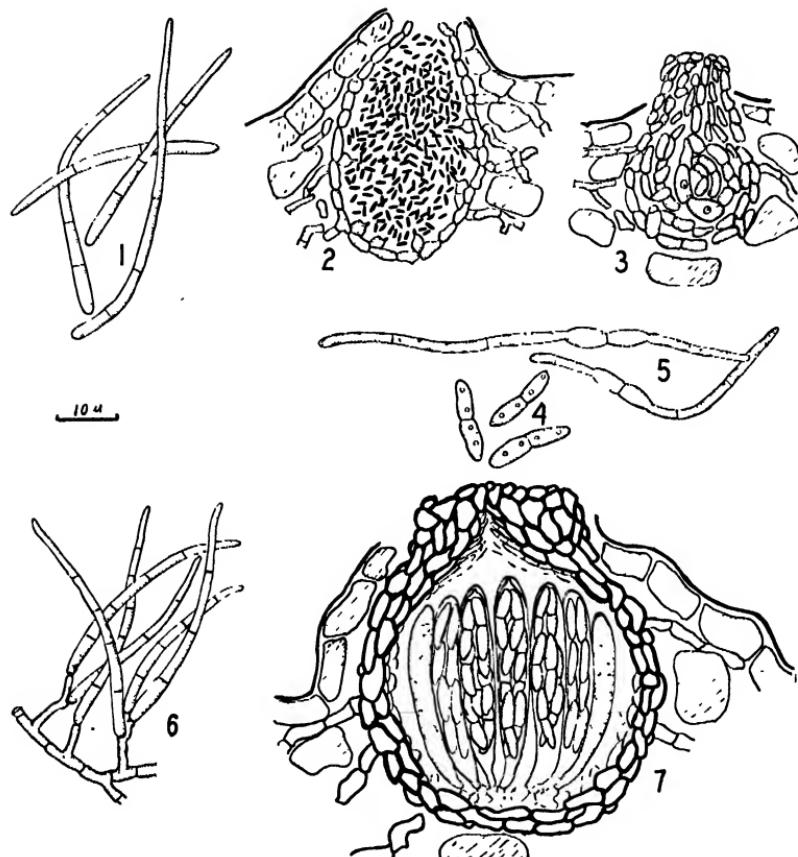
The conidial stage may be found at any time, throughout the entire summer since new lesions may appear at any time and since successive crops of conidia develop on old lesions. The lesions are characteristically large, brown, irregular leafspots (FIG. 8). They are most conspicuous on the upper leaf surface, because the covering of trichomes on the lower leaf surface renders the lesions less evident. They may, at first, be bordered with a margin that is darker brown or purplish, in which case the spots are definitely delimited, but as the surrounding uninvaded portions become pale green the margins pale out and the spots are thus less definitely delimited. The lesions may continue to enlarge until the leaves are shed by which time they may have fused and may have come to occupy most of the leaf surface. Finally the central portions of the lesions become grayish tan. They are early beset with numerous dark specks, the fascicles of conidiophores.

The conidiophores emerge from the stomates in short, small fascicles. They do not arise from a compact, extensive stroma, as occurs in many other species of *Cercospora*, although the invaded leaf tissues are occupied by an extensive, much-branched, intercellular mycelium. Conidia are formed and dislodged seriatim at the tips of the conidiophores, which become geniculate, as a result, and flexuose. The conidia (FIG. 1) are club-shaped, dilutely brown, curved, many-septate, and  $40-130 \times 3.0-4.5 \mu$ . Their length is most commonly found to be  $65-75 \mu$ .

#### SPERMOGONIA AND PERITHECIAL INITIALS

. The spermogonia and perithecial initials may be found to be present in the period extending from late August throughout

September and October. They occur on the lower surface of leaves that have recently been shed, and appear as numerous, black, pimple-like structures. Details of structure of these bodies



FIGS. 1-7. 1, conidia of *Cercospora Rubi* from lesions on leaves; 2, spermogonium in vertical section, showing pycnidial structure and spermatia; 3, stroma of perithecial initial in vertical section showing archicarp; 4, ascospores in *Mycosphaerella dubia*; 5, germinating ascospores; 6, conidia formed in culture from ascospores; 7, section of perithecioid of *Mycosphaerella dubia*, embedded within the tissues of a decaying leaf of *Rubus*.

were studied by means of sections of lesions embedded in paraffin. To do this, leaf tissues were fixed, sectioned, and stained with Haidenhain's iron alum hematoxylin. An examination of these sections shows that the spermogonia (FIG. 2) are spherical to flask-shaped conceptacles, sunken within the leaf tissues and con-

taining myriads of minute, rod-shaped spermatia. By the time that the spermogonial aperture has developed, through which the spermatia are liberated, each spermogonium projects slightly above the leaf surface. The spermogonial wall is essentially a single cell-layer in thickness. The spermatia are budded off from ampulliform, spermatiferous cells that project from the inner surface of the spermogonium.

If the lower surface of lesions is examined with low magnification, it is impossible to distinguish between spermogonia and perithecial initials. In section, however, these latter structures are found to be loosely-aggregated stromata, flask-shaped in outline, whose cells tend to be arranged in series that look like deeply constricted hyphae. Within each stroma is a hyphal coil, the archicarp (FIG. 3), whose basal cells are larger and more deeply staining than the surrounding cells. The upper portion of this coil, the trichogyne, is more slender and extends to the surface of the stroma. No direct evidence has been found, in the case of this fungus, that the trichogynes function as receptive organs or that the spermatia constitute the male elements. In the light of our knowledge of certain other Ascomycetes, however, it may reasonably be assumed that fertilization must be accomplished by the fusion of the content of a spermatium with that of the ascogone. The perithecial initial is thereafter slowly transformed into the perithecium.

#### PERITHECIAL STAGE

The perithecial stage was found to develop when leaves bearing spermogonia and perithecial initials were permitted to remain out-of-doors throughout winter. By late April or early May, the perithecia were noted to be mature in these leaves. Perithecia as seen, with low magnification, are black points, sparsely scattered over the lower leaf surface. In section, the perithecia are observed to be immersed within the decaying leaves and to project to the surface by means of short papillae. They are minute, being approximately 40–60  $\mu$  in diameter (FIG. 7). Their wall is membranous, being constituted of a thin layer of brown, rather thick-walled cells. The asci are fasciculate, and paraphyses are lacking. The ascospores are distichate, hyaline, two-celled, and

11–14  $\mu$  long, the upper cell being slightly longer and wider than the lower (FIG. 4). The ascospores increase greatly in size in the brief period immediately prior to their discharge. Apparently their size can best be determined by measurement of freshly discharged ascospores. If microscopic slides are placed above moistened leaves, bearing perithecia, the ascospores will adhere to the slides.

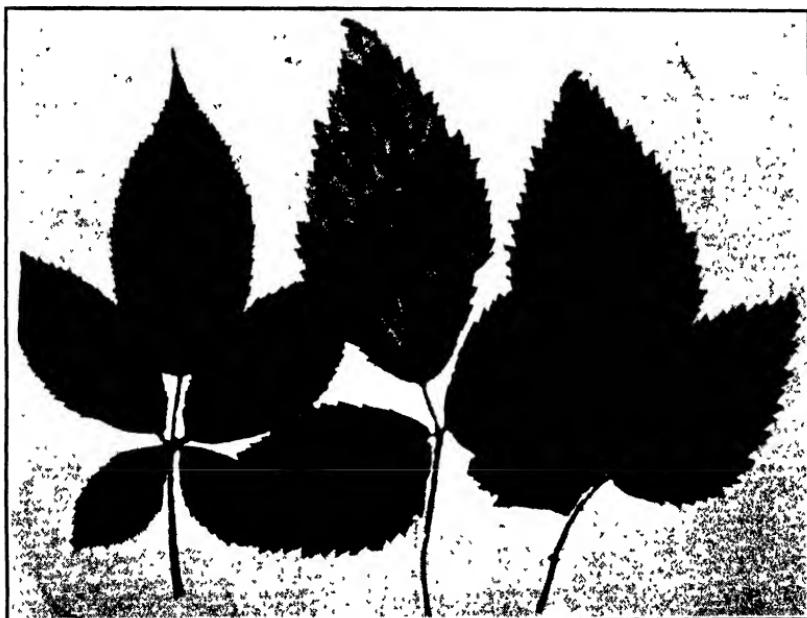


FIG. 8. Lesions on blackberry leaf (at left) and dewberry leaves (at right) produced by the conidial stage.

The morphological characteristics of the perithecial stage, just described, are clearly those of the genus *Mycosphaerella*. The genetic connection of the *Mycosphaerella* with the conidial stage was demonstrated by means of cultures. Isolations from ascospores were made by permitting the ascospores to be expelled onto the surface of corn meal agar plates. This was done by placing leaves, bearing perithecia, upon moistened blotters in the tops of Petri dishes. Agar films in the bottoms of the Petri dishes were then inverted over them. The ascospores, that were ejected, germinated by the emission of germ tubes, characteristically one from each extremity (FIG. 5). Within 72 hours, short

lateral branches, each bearing a single *Cercospora* conidium, had formed on the mycelium. These conidia were like those of *Cercospora Rubi* from leaf lesions. Within the next 2 or 3 days other conidia were abstracted from the same conidiophores, and they had become geniculate (FIG. 6). After the colonies had become more than a week old, conidia could no longer be found, and by this time the colonies had become compact, grayish mycelial mats.

Conidia taken from leaf lesions germinated on corn meal agar plates, and within a few days thereafter conidia were borne on the resulting mycelium. Furthermore the colonies that originated from conidia were entirely similar to those that arose from ascospores.

#### IDENTITY OF THE FUNGUS

The conidial stage of the fungus under consideration was first described as *Cercospora Rubi* by Saccardo (4), in 1876. His description was based upon collections on leaves of *Rubus fruticosus* from northern Italy and on leaves of *R. imperialis* from Argentina. In 1896, specimens of a fungus on *R. canadensis*, having smaller conidiophores and conidia, the conidiophores measuring  $20-25 \times 3 \mu$  and the conidia  $35-63 \times 2-2.5 \mu$ , were believed by Ellis and Everhart (2) to be distinct, and hence the fungus was named by them *Cercospora septorioides*. In 1917, a leafspot fungus on a species of blackberry from Texas, was described by Tharp (6) as *Cercospora Bliti*. An examination, by Chupp,<sup>2</sup> of type specimens of *Cercospora septorioides* and of *C. Bliti* shows that they are identical with *C. Rubi* and hence are synonymous.

The writer has not been able to compare *Cercospora Rubi* with *C. rubicola*, described by de Thümen (1), in 1881, as the cause of a leafspot of *Rubus fruticosus*, nor with *C. Garbiniana* described by Massalongo,<sup>3</sup> in 1902. Neither of these two species has been reported to occur within the United States. The lesions produced by *C. rubicola* are said to be large, ochraceous, and purple bordered, and therefore could be distinguished with difficulty, if at all, from those associated with *C. Rubi*. The conidia, how-

<sup>2</sup> Opinion communicated by letter.

<sup>3</sup> In Atti Mem. Acc. Agric., Sci. Lett, ed Arti. Verona. 3: 153, pl. 10, fig. 21, 1902.

ever, are said to be of greater diameter than those of *C. Rubi*. *C. Garbiniana* is said to differ from *C. rubicola* mainly in having reddish conidiophores and shorter, narrower conidia; and from *C. Rubi*, in producing pale, reddish brown lesions, and in possessing cylindrical, less sturdy conidia. In the light of the writer's observations on the variation in character of the lesions produced by *C. Rubi*, and on the range in size of its conidia, that overlap those given for *C. rubicola* and *C. Garbiniana*, it appears probable that both of these are also synonymous with *C. Rubi*. This remains to be established, however, by comparative studies of the types, supplemented, perhaps, by studies of their development.

Another species of *Cercospora* on *Rubus* was recently described by Sydow.<sup>4</sup> It was collected, on *Rubus rosaeifolius* Smith, in the Transvaal, and is characterized by its failure to produce definite leaf spots. Instead, an effuse, olivaceous growth of conidiophores and conidia is developed on the lower leaf surface, and this organism is unquestionably distinct from *C. Rubi*.

A survey of mycological literature dealing with *Mycosphaerella* (*Sphaerella*) on *Rubus* reveals the fact that 11 species of *Sphaerella* have been recorded to occur on species of *Rubus*. These are *Sphaerella Chamaemori* Karst., *S. fructicum* Starb., *S. idaeina* Hazsl., *S. innumerella* Karst., *S. Ligea* Sacc., *S. maculiformis* (Pers.) Auserw., *S. minoensis* Syd., *S. Rubi* Roark, *S. rubicola* McAlp., *S. rubina* Peck, and *S. Winteri* (Pass.) Sacc. An attempt was made to identify the fungus under consideration by comparison with the original descriptions of the above-named species, and by comparison with type specimens, and authoritatively determined specimens, in so far as these were available in the Farlow Herbarium or could be obtained through loans. An examination of type specimens of *Sphaerella idaeina* shows that the ascospores instead of being 14–16  $\mu$  long, as described by Hazslinsky (3), are found to range up to 26  $\mu$ . The type specimens of *S. fructicum* possess asci and ascospores of approximately the same size as the perithecial stage of *Cercospora Rubi*, but it is caulicolous. Its perithecia are of different distribution, size, and shape from the fungus under consideration, and its morphologic features are as described by Starbäck (5).

<sup>4</sup> In Ann. Myc. 22: 433–434. 1924.

The type specimens of *S. Ligea* show that it develops in irregular, brown lesions on green leaves. Moreover, *S. Ligea* is not the perfect stage of *Septoria Rubi* Westd., as suggested by Saccardo. The writer is familiar with *Septoria Rubi* and with its perfect stage, *Mycosphaerella Rubi* Roark, both of which stages he has collected and observed.

Measurements of *S. Winteri* are not given in Saccardo's *Sylloge Fungorum* but an examination of authoritatively determined specimens from Hungary shows that its perithecia arise within large, concentrically zonate spots on green leaves and that it is considerably larger than the perithecial stage of *Cercospora Rubi*.

The ascospores of *Sphaerella Chamaemori*, *S. innumerella*, *S. minoensis*, or *S. rubicola* are approximately twice as long as those of the fungus with which the writer is dealing. *Sphaerella maculiformis* occurs on the decaying leaves of a variety of woody plants including oaks, chestnut, sycamore, and basswood. Although its ascospores correspond closely in size with the fungus under consideration, its perithecia and asci are much larger. There does not appear to be any likelihood that it is identical with the fungus whose conidial stage is *Cercospora Rubi* since these trees are not attacked by a fungus like *Cercospora Rubi*. The perithecia of *S. rubina* are approximately four times as large, the asci twice as large, and the ascospores twice as wide as those of the fungus under consideration.

It would appear unlikely that the perfect stage of a leafspot fungus that is as commonly prevalent and as widely distributed as this one on *Rubus* could have escaped being collected and described previously. It would also appear equally improbable that 11 species of *Sphaerella* could occur on *Rubus*. Certain of them appear to the writer to be synonymous, but he hesitates to assign any of them to synonymy without first having made a detailed study of their morphology and cycle of development. Neither has he been able to establish beyond a reasonable doubt that the organism under consideration is or is not one of the 11 so-called species of *Sphaerella* on *Rubus*. The problem of identity and synonymy of species of *Sphaerella* on *Rubus* must remain for some future investigation. It is believed, however, that least confusion would result, at this time, because the fungus under

consideration is so widely known in its conidial stage, and its identity could thus readily be established, if a new specific name were erected. It is proposed therefore to assign a new name to the fungus whose conidial stage is *Cercospora Rubi*, and it is briefly described as follows:

**Mycosphaerella dubia** sp. nov.

Syn. *Cercospora Rubi* Sacc. Nuovo Giorn. Bot. Ital. 8: 188.  
1876.

*Cercospora septorioides* Ellis & Ev. Publ. Field Mus. Bot. 1: 94. 1896.

*Cercospora Bliti* Tharp, Jour. Myc. 9: 108. 1917.

Peritheciis sparsis, hypophyllis, sphaeroideis, ostiolo papillaeformi praeditis, semi-immersis deinde erumpentibus, nigris, 40–60  $\mu$  diam.; ascis fasciculatis, clavatis, brevistipitatis, a paraphysatis, 8-sporis, apice tunica incrassatis; sporidiis bi-seriatibus, hyalinis vel subhyalis, curvatis, oblongo-cylindricis, bilocularibus, loculis inaequalibus, quorum alter vel angustior vel latior, alter est longior, 11–14  $\mu$  longis, crassites sporidiorum est variabilis.

Spermogoniis autumno efformantibus, hypophyllis, innato erumpentibus, punctiformibus, sparsis, nigris, globosis, 20–25  $\mu$  diam.; spermatiis bacillaribus, hyalinis, 2–3  $\times$  1–1.5  $\mu$ .

Hab. in foliis dejectis *Rubi* spp.

Status conidicus: Maculis aridis, brunneis, deinde griseolis, magnis, subcircularibus vel irregularibus, saepe confluentibus; hyphis epiphyllis, fuscis, stomatibus pertussis, sursum geniculato-flexuosis; conidiis longe clavulatis, sursum attenuatis, curvulis 40–130  $\times$  3–4.5  $\mu$ , pluriseptatis, subhyalis vel dilute fuscis.

Hab. ad folia viva *Rubi* spp., frequens.

For the convenience of mycologists type specimens have been deposited in the Farlow Herbarium, Harvard University, Cambridge, Mass. Co-type specimens have been deposited in the Herbarium of Mycological Collections, U. S. Dept. of Agriculture, Washington, D. C., the Hungarian National Museum, Budapest, Hungary, and the Naturhistoriska Riksmusset, Stockholm, Sweden.

#### SUMMARY

This investigation deals with the morphology and development of a common leafspot fungus of many species of *Rubus* that is known in its conidial stage as *Cercospora Rubi* Sacc.

This fungus has been found to possess a perithecial stage that is initiated in autumn by the formation of spermogonia and archicarps. The perithecia mature in spring.

Genetic connection of the conidial and perithecial stages has been established by growth in culture.

The conidial stage is identical with *Cercospora septorioides* Ellis & Ev. and *C. Bliti* Tharp., both of which names are assigned to synonymy. *C. rubicola* and *C. Garbiniana* may also be synonymous.

The perithecial stage cannot be identified with previously described species of *Sphaerella* on *Rubus* and is herein briefly described as *Mycosphaerella dubia*.

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# THE HYDNACEAE OF IOWA IV. THE GENERA STECCHERINUM, AURISCALPIUM, HERICIUM, DENTINUM AND CALODON

L. W. MILLER

(WITH PLATE 33)

The pileate members of the Hydnaceae of North America have been carefully studied and treated by Dr. Howard J. Bunker. His paper, "A contribution to a revision of the North American Hydnaceae" (1906), and the type studies which appeared in a number of papers from 1912 to 1914 are particularly valuable. Additional knowledge has been contributed by Coker, Lloyd, Beardslee and others. With a few exceptions Bunker's conception of the genera and species is followed, and only such changes are made as seem to the writer desirable or in accord with more recent rules of botanical nomenclature.

STECCHERINUM S. F. Gray, Nat. Arr. Brit. Pl. 1: 651. 1821

Pileus reflexed to laterally substipitate, or entirely resupinate, tough or occasionally subfleshy; spores minute, smooth, ovoid to oblong, hyaline. Growing on wood.

## KEY TO THE SPECIES OF STECCHERINUM

1. Mostly resupinate, sometimes reflexed or rarely distinctly pileate; with numerous, incrusted, elongated, cylindrical or clavate and emerging cystidia.....(2)
1. Distinctly pileate, rarely reflexed; cystidia, if present, short, fusiform and relatively few in number.....(4)
2. Resupinate, sodden, corky-fibrous; spines often crowded and coalesced at the base; spores  $6-7 \times 3-4 \mu$ .....5. *S. setulosum*.
2. Resupinate, reflexed or rarely entirely pileate, dry, soft, leathery; spines generally free; spores  $3-5.5 \times 2-3.5 \mu$ .....(3)
3. Margin entire, soft; spines terete or angular, more or less uniform in shape and size; seldom darker than vinaceous cinnamon; spores  $3-4 \times 2-2.5 \mu$ .....1. *S. ochraceum*.
3. Margin radially and irregularly fimbriate; spines terete or greatly flattened, often serrate, irregular in size; more orange than vinaceous cinnamon; spores  $4-5.5 \times 3-3.5 \mu$ .....2. *S. lacticolor*.
4. Spines 6-18 mm. in length; cystidia short, fusiform; spores  $4-6 \times 2.5-3.5 \mu$ .....3. *S. septentrionale*.

4. Spines 1–5 mm. in length; cystidia fusiform or absent; spores 5  $\mu$  or less in length.....(5)
5. Texture soft, fibrous-fleshy; cystidia absent; hyphae with 2 prominent clamp connections at many septa; spores 4–5  $\times$  2–2.5  $\mu$ .
  4. *S. pulcherrimum*.
5. Texture leathery, fibrous; cystidia present or absent; clamp connections single or absent; spores 4  $\mu$  or less in length.....(6)
6. Faintly zoned near the margin only; spores short cylindrical, 2.5–3  $\times$  1–1.25  $\mu$ .....6. *S. adustum*.
6. Distinctly zonate; spores ovoid to ellipsoid.....(7)
7. Cystidia fusiform; spores ellipsoid, 3–4  $\times$  2  $\mu$ .....7. *S. rawakense*.
7. Cystidia absent; spores ovoid, 2–2.5  $\times$  1.5  $\mu$ .....8. *S. pusillum*.

1. STECCHERINUM OCHRACEUM (Fries) S. F. Gr<sup>ay</sup>, Nat. Arr. Brit. Pl. 1: 651. 1821. (FIG. 1)

*Hydnnum ochraceum* Pers. ex Fries, Syst. Myc. 1: 414. 1821.

*Hydnnum Rhois* Schw. Schr. Nat. Gaz. Leipzig 1: 103. 1822.

*Hydnnum pudorinum* Fries, Elench. 1: 133. 1828.

*Hydnnum flabelliforme* Berk. Lond. Jour. Bot. 4: 306. 1845.

*Hydnnum plumarium* Berk. & Curt. Grevillea 1: 97. 1873.

*Climacodon ochraceus* (Fries) Karst. Bidr. Finl. Nat. Folk. 37: 98. 1882.

*Climacodon pudorinus* (Fries) Karst. Bidr. Finl. Nat. Folk. 37: 97. 1882.

*Leptodon pudorinum* Quél. Fl. Myc. Fr. 440. 1888.

*Hydnnum conchiforme* Sacc. Syll. Fung. 6: 458. 1888.

*Mycoleptodon ochraceum* Pat. Tax. Hymén. 116. 1900.

*Steccherinum Rhois* (Schw.) Bunker, Mem. Torrey Club 12: 126. 1906.

*Hydnnum alnicolum* Vel. České houby. 745. 1922.

Fructification resupinate, reflexed or entirely pileate and laterally sessile, fibrous, tough, slightly separable, the upper surface of the pileus soft, tomentose, sulcate zoned, cartridge buff; hymenial surface pinkish cinnamon to vinaceous cinnamon; margin whitish, pubescent, subfimbriate, usually wider in the resupinate portions than in the reflexed; spines 2 mm. or less in length, crowded, 4–6 per mm., slender, subulate, pointed, terete or flattened, often divided, gradually decreasing in length toward the margin; hyphae thick-walled, 2–6  $\mu$  in diameter, coarser and less compactly interwoven next to the substratum or on the upper surface of the pileus, with scattered clamp connections, thin-walled in the sub-hymenial region and not exceeding 3.5  $\mu$  in diameter; cystidia 20–100  $\times$  4–9  $\mu$ , numerous, projecting singly

from the sides and the apex of the spines, elongated, clavate to cylindrical, slightly curved, arising from the parallel hyphae in the axial portion of the spine, thick-walled, usually heavily incrusted; basidia  $12-15 \times 3-5 \mu$ , clavate; spores  $3-4 \times 2-2.5 \mu$ , obovate, smooth, hyaline.

This species is recognized by its tough texture, its ochraceous color, the numerous elongated and projecting cystidia and the small, obovate spores. It varies much in habit of growth. *Hydnnum flabelliforme* Berk. and *Hydnnum Rhois* Schw. seem to refer to the more pileate variations of the species.

Abundant in Iowa on dead wood of deciduous trees; collected in all seasons. Apparently common throughout the United States.

2. STECCHERINUM LAETICOLOR (Berk. & Curt.) Banker, Mycologia 4: 316. 1912

*Hydnnum laeticolor* Berk. & Curt. Grevillea 1: 99. 1873.

*Irpex laeticolor* (Berk. & Curt.) Morgan, Jour. Cinc. Soc. Nat. Hist. 10: 15. 1887.

*Mycoleptodon laeticolor* (Berk. & Curt.) Pat. Tax. Hymén. 116. 1900.

Resupinate or with a slightly detached or reflexed margin, soft spongy and slightly tough in texture, somewhat separable, zinc orange to orange-cinnamon; margin floccose, radially fimbriate, white or lighter in color; spines 2 mm. or less in length, irregular, flattened, serrate or divided, Irpex-like, obtuse; hyphae  $3-6 \mu$  in diameter, thick-walled, with scattered clamp connections; cystidia  $5-9 \mu$  in diameter, cylindrical, elongated, incrusted, projecting at the sides and apex of the spines; basidia  $8-15 \times 3-5 \mu$ , clavate; spores  $4-5.5 \times 3-3.5 \mu$ , ellipsoid, smooth, hyaline.

*S. laeticolor* closely resembles *S. ochraceum*. It may be separated in most cases by the more reddish color, the soft texture, the radially fimbriate margin, the irregular teeth and the larger spores. The larger spores are diagnostic. A fragment of the type at the New York Botanical Garden has been examined.

Uncommon in Iowa; collected in August and February. Widely reported from the central and eastern United States. Apparently more common in the southern states.

3. STECCHERINUM SEPTENTRIONALE (Fries) Banker, Mem. Torrey Club 12: 130. 1906. (FIG. 3)

*Hydnus septentrionale* Fries, Syst. Myc. 1: 414. 1821.

*Climacodon septentrionalis* Karst. Rev. Myc. 3: 20. 1881.

*Creolophus septentrionalis* (Fries) Banker, Mycologia 5: 293. 1913.

Fructification tough, fibrous, generally consisting of many sessile, horizontal pilei which are imbricate, confluent at the base and arising from a small, single point of attachment, reported to weigh as much as 60 lbs. (Lloyd); single pilei more or less flattened, usually 3–15 cm. wide, 2–15 cm. long, reported up to 30 cm. wide and 20 cm. long, fibrous and pubescent on the upper surface, not zoned, reported white, becoming coarsely wrinkled and ochraceous buff in the herbarium; margin abrupt, slightly incurved; spines 6–18 mm. in length, 2–3 per mm., slender, terete, pointed, entire, reported milk-white, becoming prussian red to pecan brown in the herbarium; hyphae 3–6  $\mu$  in diameter, compactly interwoven, often agglutinated, with few clamp connections, usually accompanied by larger, scattered and apparently gelatinized hyphae measuring 6–11  $\mu$  in diameter; cystidia 30–50  $\times$  10–16  $\mu$ , short, fusiform, thick-walled, projecting 10–25  $\mu$ ; basidia 12–18  $\times$  4–5  $\mu$ , clavate; spores 4–6  $\times$  2.5–3.5  $\mu$ , ellipsoid, smooth, hyaline.

*S. septentrionale* is similar to *S. pulcherrimum* and the two have often been confused. The former species, however, is dryer, tougher and remains a paler color in the herbarium. This species may be distinguished also microscopically by the presence of the fusiform cystidia and the absence of the two clamp connections at the septa of hyphae. The many horizontal and usually joined pilei are very characteristic for the species.

Unfortunately I have been unable to see a living specimen of *S. septentrionale*. The characters noted above for fresh fructifications have been taken largely from the description of Banker. I find a considerably greater range in the length of the spine. Several specimens quite obviously correctly determined have spines not exceeding 6 mm. in length. Banker reports the spines as 17–18 mm. in length.

Collected on maple in August; not common in Iowa. Apparently occurs throughout the central and the eastern United States.

4. **STECCHERINUM PULCHERRIMUM** (Berk. & Curt.) Banker, Mem.  
Torrey Club 12: 129. 1906. (FIG. 7)

*Hydnnum pulcherrimum* Berk. & Curt. Hooker's Jour. Bot. and  
Kew Gard. Misc. 1: 235. 1849.

*Hydnnum friabile* Fries, Nova Acta Soc. Sci. Upsal. III. 1: 106.  
1851.

*Steccherinum agaricoides* (Swartz) Banker, Mem. Torrey Club 12:  
130. 1906.

*Creolophus pulcherrimus* (Berk. & Curt.) Banker, Mycologia 5:  
294. 1913.

*Creolophus agaricoides* (Swartz) Banker, Mycologia 5: 294. 1913.

Fructification pileate, sessile, solitary to imbricate, dimidiate to flabelliform, often arising from a resupinate base, sometimes bell-shaped or convex, variable in size, soft, pliable, somewhat tough, fibrous, juicy when fresh and sometimes containing a milky white, sticky sap, soft and gummy to brittle when dry, whitish at first becoming light buff, tawny to liver brown in the herbarium; individual pilei up to 15 cm. in width and 8 cm. in length, 0.2–10 mm. thick at the base, densely fibrous or hairy on the upper surface; margin very thin; spines 1–6 mm. in length, usually about 5 mm., 4–5 per mm., slender, terete, pointed, entire, tawny in herbarium; hyphae 4–6  $\mu$  in diameter, loosely packed in the pileus, with slightly thickened walls, with numerous clamp connections, often two at each septum, more fragile and with fewer clamp connections in the spines, 2–3  $\mu$  in diameter; basidia 12–18  $\times$  3–4  $\mu$ , clavate; spores 4–5  $\times$  2–2.5  $\mu$ , variable, ellipsoid, slightly depressed laterally, smooth, hyaline.

The soft, somewhat fibrous texture, the tawny color, the white, sticky sap when fresh and the two clamp connections at many of the septa of the coarse hyphae are well marked characters in this species. These double clamp connections are sometimes few in number but I have never failed to find them in the several dozen specimens examined. I have examined, at The New York Botanical Garden, the *Murrill* and *Harris* specimen, No. 1095, which Banker regards as representing *Hydnnum agaricoides* Swartz. It seems to be a young growth of *S. pulcherrimum* and agrees in every microscopic detail with a typical fructification. It varies only in being slightly more fleshy and has short undeveloped teeth.

Rather uncommon in Iowa, occurring on dead wood of various

frondose species from August to September. The seasonal range is probably greater than reported. Only two specimens in our herbarium have the date given. Widely reported from the eastern half of the United States.

5. **Steccherinum setulosum** (Berk. & Curt.) comb. nov. (FIG. 2)  
*Hydnnum setulosum* Berk. & Curt. Grevillea 1: 100. 1873.

Resupinate, effused, adnate, thick, soft corky-fibrous, waterlogged when fresh, avellaneous, contracting and curling upon drying but not cracking, cinnamon to army brown in the herbarium; margin concolor; spines 2-9 mm. in length; variable, usually crowded and coalesced more or less at the base, terete, cylindrical and obtuse to subulate and acute, sometimes flattened and branched; hyphae 1.5-4  $\mu$  in diameter, thin-walled, with scattered clamp connections, others thick-walled, compactly arranged in older fructifications; cystidia 30-90  $\times$  4-10  $\mu$ , numerous, long, cylindrical to clavate, obtuse, incrusted, arising from the thick-walled hyphae in the axial portion of the spine and in the subiculum, projecting; basidia 20-25  $\times$  6-7  $\mu$ , clavate, with 4 sterig mata; spores 6-7  $\times$  3-4  $\mu$ , ellipsoid, smooth, granular or guttulate.

*S. setulosum* is characterized by the sodden, soft fibrous texture, the rather long, more or less coalesced spines, the numerous, elongated cystidia and the large spores. This species received its name because of the numerous cystidia projecting uniformly from the sides of the spines, a character readily detected under a lens.

A minute fragment of the type of *Hydnnum setulosum* was examined at The New York Botanical Garden. It is characterized by smaller and less crowded spines than in Iowa specimens and according to the original description differs also in the possession of a broad sterile border. Iowa specimens were identical in color, texture and microscopic structure. An Iowa specimen was sent to Kew in order that it might be compared with a larger portion of the original specimen. Miss Wakefield reports that it agrees with the type.

Collected in September and October on dead wood and an old sporophore of *Fomes*. Relatively uncommon in Iowa. Apparently reported only from the type locality in Alabama.

6. **STECCHERINUM ADUSTUM** (Schw.) Banker, Mem. Torrey Club  
12: 132. 1906. (FIG. 4)

*Hydnnum adustum* Schw. Schr. Nat. Ges. Leipzig 1: 103. 1822.

Pileus tough, fibrous, brittle in the herbarium, dimidiate, flabelliform or reniform, with a resupinate base, laterally sessile or more often with a short lateral stipe, occasionally with an eccentric or central stipe, often subimbricate with abortive or fully formed pilei arising from the upper surface of lower ones, 3-7 cm. wide, 2-4 cm. long, more or less flattened, depressed toward the substratum or stipe, with a finely pubescent, slightly duller than cinnamon buff surface, faintly zoned toward the margin; spines 1-3 mm. in length, 4-5 per mm., slender, terete, angular or flattened, sometimes coalescing, often divided at the apex, whitish at first, becoming russet in the herbarium; hyphae 2-5  $\mu$  in diameter, quite homogeneous in the pileus, thick-walled in the pileus and axial portion of the spines, with scattered clamp connections; basidia 8-15  $\times$  3  $\mu$ , clavate; spores 2.5-3  $\times$  1-1.25  $\mu$ , minute, short cylindrical, smooth, hyaline or granular.

This species may be recognized by the more or less dry, tough, reniform pileus which usually has a short, stout, lateral stipe and by the minute spores. *Steccherinum pusillum* (Brot.) Banker is similar but may be separated by the distinctly zonate pileus and the ovoid spores.

Collected in Iowa on dead wood from July to November. Not common. Apparently widely distributed in the eastern United States and Canada.

7. **STECCHERINUM RAWAKENSE** (Pers.) Banker, Mycologia 4:  
312. 1912. (FIG. 5)

*Hydnnum rawakense* Pers. Freyc. Voy. Aut. Monf. Bot. 175. 1826.

*Hydnnum reniforme* Berk. & Curt. Jour. Linn. Soc. 10: 325. 1869.

*Hydnnum glabrescens* Berk. & Rav. Grevillea 1: 97. 1873.

*Hydnnum guaraniticum* Speg. Anal. Soc. Cl. Argent. 17: 74. 1884.

*Hydnnum basiasperatum* P. Henn. Hedwigia 36: 199. 1897.

*Steccherinum Morgani* Banker, Mem. Torrey Club 12: 127. 1906.

*Steccherinum reniforme* (Berk. & Curt.) Banker, Mem. Torrey Club 12: 127. 1906.

Pileus tough, fibrous, brittle in the herbarium, horizontal, reniform to flabelliform, more or less flattened, laterally sessile or substipitate, occasionally with a small, resupinate base, closely

imbricate, often confluent toward the base; upper surface sulcate-zonate, glabrous or subpubescent with very short, coarse hairs, cinnamon in the herbarium; spines 2.5 mm. or less in length, 4–5 per mm., slender, terete or angular, subulate, pointed, chestnut-brown in the herbarium; hyphae 2.5–6  $\mu$  in diameter, thick-walled in the pileus and the axial portion of the spines, without clamp connections; cystidia 15–30  $\times$  5–9  $\mu$ , short fusiform, pointed thick-walled, entirely hymenial, projecting about 10  $\mu$ ; basidia 12–15  $\times$  4  $\mu$ , clavate; spores 3–4  $\times$  2  $\mu$ , ellipsoid, smooth, hyaline.

This species resembles *Steccherinum adustum* in size, texture and general appearance but the pileus is usually less stipitate and more distinctly zoned, the spores are larger and cystidia are present. The cystidia and larger spores distinguish this species also from *Steccherinum pusillum* (Brot.) Banker. I have examined the specimen at The New York Botanical Garden referred by Morgan to *Hydnnum glabrescens* Berk. & Rav. and upon which Banker erected *Steccherinum Morgani*. It is identical in microscopic detail with *Steccherinum rawakense* (Pers.) as that species is understood by Banker. The slight differences in the external characters of this specimen from the typical condition in *S. rawakense* do not seem sufficient grounds for separating it as a distinct species. The type of *Hydnnum glabrescens* was also studied at The New York Botanical Garden. The original description of *Hydnnum rawakense* Pers. has not been seen. The citation is taken from Banker.

The single specimen in the University of Iowa herbarium was collected in Johnson County, Iowa, in November. Rare. Reported from several scattered localities in the eastern United States.

8. STECCHERINUM PUSILLUM Brot. ex Banker, Mycologia 4: 313.  
1912. (FIG. 6)

*Steccherinum adustulum* Banker, Mem. Torrey Club 12: 133.  
1906.

Pileus tough, fibrous, brittle in the herbarium, laterally stipitate or sessile, reniform or somewhat irregular, whitish with slightly darker zones on the upper surface; spines 2 mm. or less in length, 5–7 per mm., slender, flexuous, terete to flattened; cystidia absent; hyphae 2.5–4  $\mu$  in diameter, thick-walled, with-

out clamp connections; basidia  $10-15 \times 3.5-5 \mu$ , clavate; spores  $2-2.5 \times 1.5 \mu$ , ovoid, smooth, hyaline.

*S. pusillum* is reported from Iowa but no specimen believed to represent this species was found in the University of Iowa herbarium. The above notes were taken from a study of the type of *S. adustulum* at The New York Botanical Garden. This species seems closely related to *S. rawakense* and *S. adustum*.

**AURISCALPIUM** S. F. Gray, Nat. Arr. Brit. Pl. 1: 650. 1821

Pileus entire or lobed, laterally stipitate, leathery; spines slender, subulate, spores hyaline, small. Growing on cones of conifers.

**AURISCALPIUM VULGARE** S. F. Gray, Nat. Arr. Brit. Pl. 1: 650. 1821. (FIG. 10)

*Hydnnum Auriscalpium* L. ex Fries, Syst. Myc. 1: 406. 1821.

*Pleurodon Auriscalpium* (Fries) Quél. in Cooke & Quélet, Clav. Hymen. 198. 1878.

*Auriscalpium vulgare* Karst. Medd. Soc. Faun. Fl. Fenn. 5: 27. 1879.

*Leptodon Auriscalpium* Quél. Ench. Fung. 192. 1886.

*Auriscalpium Auriscalpium* (Fries) Bunker, Mem. Torrey Club 12: 178. 1906.

Pileus 1-2 cm. wide, horizontal, cordate to reniform, supported laterally, slightly convex, leathery, finely hispid, bister to almost black; stipe vertical, 1-6 cm. long, slender, leathery, finely hispid, bister to almost black in upper portion, swollen, spongy and a lighter shade of brown at the base; spines 1.5 mm. or less in length, 4-5 per mm., slender, terete, acute; hyphae 2-6  $\mu$ . with thickened walls, few clamp connections, faintly colored, compactly arranged, fascicled to some extent in the pileus and the stipe; cystidia occasionally present,  $15-30 \times 3-6 \mu$ ; thin-walled, subulate; basidia  $15-20 \times 5-6 \mu$ , clavate, with 2-4 sterigmata; spores  $4.5-5 \times 3.5-4 \mu$ , obovate, apiculate or slightly attenuated at the base, smooth or minutely papillose, hyaline.

This species is recognized by the slender, vertical stipe, the reniform, laterally supported pileus, the dark color and hispid character of the stipe and pileus.

On decaying cones of coniferous species; June to November. Rare in Iowa. Reported from Oregon, Arizona and from various localities in central and eastern United States.

**HERICIUM** Pers. ex S. F. Gray, Nat. Arr. Brit. Pl. 1: 652. 1821

Fleshy or subfleshy, pulvinate or branched; spines long, pendent; gloecystidia usually present; spores subspherical, guttulate. Growing on wood.

KEY TO THE SPECIES OF HERICIUM

1. Fructification consisting of a mass of branching processes; spores  $4-7 \mu$  in diameter.....(2)
1. Fructification massive, rarely showing a tendency to form branching processes; spines long, more or less uniform in size and distribution, 1-4 cm. in length.....3. *H. Erinaceus*.
2. Intricately branched and anastomosing; spines seldom over 6 mm. in length, more or less uniformly distributed on the underside of the slender branches; spores  $4-5 \times 3-4 \mu$  in diameter..1. *H. laciniatum*.
2. Main branches few, short, relatively stout; spines 5-15 mm. in length, typically in fascicles at the ends of the terminal branches or on short lateral spurs; spores  $5.5-7 \mu$  in diameter.....2. *H. coralloides*.

1. **HERICIUM LACINIATUM** Leers ex Banker, Mem. Torrey Club 12: 114. 1906. (FIG. 8)

*Hydnum coralloides* Scop. ex Fries, Syst. Myc. 1: 408, in part. 1821.

*Medusina coralloides* Chev. Fl. Gen. Env. Paris 1: 279. 1826.

*Manina flagellum* Scop. ex Banker, Mycologia 4: 276. 1912.

Fructification consisting of a repeatedly dividing and more or less anastomosing mass of branches, arising from a common base which usually penetrates the substratum, soft, fleshy, whitish, becoming cream-buff to russet in the herbarium; spines 1-6 mm. in length, terete, subulate, acute, more or less uniformly distributed on the under side of the branches, often borne on the upper side of the terminal branchlets; hyphae  $3-20 \mu$  in diameter, with occasional clamp connections; gloecystidia  $5-7 \mu$  in diameter, arising from the subhymenial region and penetrating the hymenium, containing a yellowish, refractive material; basidia  $20-35 \times 5-6 \mu$ , clavate, slightly guttulate; spores  $4-5 \times 3-4 \mu$ , smooth, hyaline, 1-guttulate.

This species is recognized by the characteristic fructification. It is more intricately branched than *H. coralloides*, has shorter and more uniformly distributed spines and smaller spores. According to Banker (1906 & 1912) this species was named *Hydnum flagellum* by Scopoli (1772), *Hydnum laciniatum* by Leers (1775), and *Hydnum ramosum* by Bulliard (1791) and is distinct from *H. coralloides* Scop. The pre-Friesian synonymy has not been

checked. Fries in the *Systema Mycologicum* considered Leers and Bulliard's names as synonyms of *Hydnus coralloides* Scop., the viewpoint followed by most mycologists since the time of Fries. I have examined a considerable number of specimens of *coralloides* Scop. from Europe and America and it seems apparent, as Bunker states, that the forms commonly included under this name represent two valid species.

Common in Iowa on dead logs of frondose species from August to November. Widely reported from the United States. Probably more generally known as *Hydnus coralloides*.

2. HERICIUM CORALLOIDES Scop. ex S. F. Gray, Nat. Arr. Brit. Pl. 1: 652. 1821. (FIG. 14)

*Hydnus coralloides* Scop. ex Fries, Syst. Myc. 1: 408, in part. 1821.

*Dryodon coralloides* (Fries) Quél. in Cooke & Quél. Clav. Hymen. 198. 1878.

*Friesites coralloides* (Fries) Karst. Medd. Soc. Faun. Fl. Fenn. 5: 27. 1879.

Fructification usually consisting of several relatively short, stout, main branches from which short and more slender terminal branches arise, soft, fleshy, whitish, becoming warm-buff to various shades of brown in the herbarium; spines 5–15 mm. in length, terete, acute, usually arising in terminal clusters or on short lateral nodules from the sides of the main branches; hyphae 3–20  $\mu$  in diameter, with occasional clamp connections; gloeo-cystidia 6–8  $\mu$  in diameter, sometimes emerging up to 25  $\mu$ ; basidia 20–45  $\times$  5–6  $\mu$ , clavate; spores 5.5–7  $\mu$ , spherical, smooth, hyaline, 1-guttulate.

*Hericium coralloides* merges gradually into the form commonly known as *H. Caput-ursi*. Whether the two should be regarded as distinct is uncertain. I have examined a great many specimens of both species at The New York Botanical Garden and in other herbaria and am unable to distinguish the two by microscopic characters. A gradual series may be built up from one to the other. The two extremes of the series are quite different in general appearance. The branching fructification of *Hericium coralloides* resembles closely *H. laciniatum* but may be separated at once by the larger spores. The two have often been confused. Out of ten specimens of *H. coralloides* examined at The New York

Botanical Garden from ten European mycologists, six were *H. coralloides* as here defined and four were *H. laciniatum*. The species concepts of *H. coralloides* and *H. laciniatum* as here employed are those of Bunker.

Collected from August to October. Uncommon in Iowa. Widely reported from the United States. Many of these reports undoubtedly refer to *H. laciniatum*.

3. HERICIUM ERINACEUS (Fries) Pers. Myc. Eu. 2: 153. 1825.  
(FIG. 13)

*Hydnnum Erinaceus* Bull. ex Fries, Syst. Myc. 1: 407. 1821.

*Medusina patula* Chev. Gen. Env. Paris 1: 279. 1826.

*Dryodon Erinaceus* (Fries) Quél. in Cooke & Quél. Clav. Hymen. 198. 1878.

*Manina cordiformis* Scop. ex Bunker, Mycologia 4: 277. 1912.

Fructification a solid or porous, slightly flattened, massive body, laterally attached, bearing on the lower and outer portions long, downward-directed spines, soft fleshy, whitish, becoming warm-buff to various shades of brown in the herbarium; spines 1–4 cm. long, curved, terete, acute, more or less coalesced at the base, often merging to shorter, flexuous and sterile spines or hairs on the upper surface; hyphae 3–20  $\mu$  in diameter, with occasional clamp connections; gloeocystidia 5–9  $\mu$  in diameter, arising from the subhymenial region, conspicuous by the highly refractive content; basidia 25–40  $\times$  5–7  $\mu$ , with 4 sterigmata; spores 5–6.5  $\times$  4.5–5  $\mu$ , subspherical, smooth, hyaline, 1-guttulate.

The massive fructification with the long cylindrical spines clearly distinguishes this species. *Hydnnum Caput-medusae* Bull. ex Fries is believed to represent a variation in which deformed spines are more apparent on the upper surface and the massive body shows some tendency of being made up of dividing and anastomosing processes.

Common in Iowa on living oak trees and dead wood of various frondose species from May to November. Widely reported from the United States.

DENTINUM S. F. Gray, Nat. Arr. Brit. Pl. 1: 650. 1821

Pileus with a central stipe, fleshy, white or pale; spores subspherical, white in mass. Growing on the ground.

**DENTINUM REPANDUM** (Fries) S. F. Gray, Nat. Arr. Brit. Pl. 1:  
650. 1821. (FIG. 9)

*Hydnus repandum* L. ex Fries, Syst. Myc. 1: 400. 1821.

*Tyrodon repandus* (Fries) Karst. Rev. Myc. 3: 19. 1881.

*Sarcodon repandum* (Fries) Quél. in Cooke & Quél. Clav. Hymen. 196. 1878.

*Hydnus umbilicatum* Peck, Bull. N. Y. State Mus. 10: 953.  
1902.

*Hypothele repanda* (Fries) Banker, Torreya 4: 113. 1904.

Pileus up to 12 cm. in diameter, usually convex, sometimes depressed, irregular, uneven, fleshy, soft, cartridge buff to pinkish cinnamon when fresh, becoming warm buff to cinnamon-buff and wrinkled when dry, with slight odor and taste, gregarious; margin repand; stipe smooth, solid, 7 cm. or less in length, color and texture as in pileus; spines 1–6 mm. in length, 2–4 per mm., soft, fleshy, subulate to occasionally flattened, slightly decurrent as short warts; hyphae 3–12  $\mu$  in diameter, thin-walled, with scattered clamp connections; basidia 25–40  $\times$  5–9  $\mu$ , clavate, with 4 sterigmata; spores 7–9  $\times$  6.5–7.5  $\mu$ , obovate to subspherical, with a prominent apiculus, smooth, white in mass.

*Hydnus repandum* is recognized by the pallid, fleshy fructification which occurs strictly on the ground, and by the large obovate spores. It varies considerably in size, color and character of the spines.

Collected from August to October on the ground; common in Iowa. Its occurrence is widely reported throughout the United States.

**CALODON** Quél. in Cooke & Quél. Clav. Hymen. 196. 1878

With central stipe, fibrous, tough, dark colored; spores subspherical, echinulate to coarsely angular, subhyaline to brown. Growing on the ground.

#### KEY TO THE SPECIES OF CALODON

1. Pileus and teeth white to grayish brown; spores subhyaline.
  1. *C. amicum*.  
Pileus and teeth darker; spores distinctly brown.....(2)
  2. Pileus mostly convex or plane, not zoned, composed of two thick and very conspicuous layers, a spongy upper and a hard, compact lower layer; spores 5–6.5  $\mu$  in diameter.....5. *C. velutinum*.
  2. Pileus depressed in the center or infundibular, usually more or less zonate, duplex texture absent or less pronounced; spores 4.5–6  $\times$  3.5–4.5  $\mu$ .....(3)

3. Pileus distinctly zonate, occasionally roughened, without a reddish liquid.  
     2. *C. zonatum*.
3. Pileus weakly zoned; more roughened.....(4)  
     4. Surface finely pubescent; with a reddish liquid.....3. *C. ferrugineum*.  
     4. Surface glabrous, without a reddish liquid.....4. *C. scrobiculatum*.
1. CALODON AMICUM Quél. Assoc. Fr. Av. Sci. Compte Rendu  
     12: 504. 1884. (FIG. 11)

*Hydnnum amicum* Quél. Grevillea 8: 115. 1880.

*Hydnnum vellereum* Peck, Ann. Rep. N. Y. State Mus. 50: 110.  
     1897.

*Phellodon vellereus* (Peck) Banker, Mem. Torrey Club 12: 168.  
     1906.

*Phellodon amicus* (Quél.) Banker, Mycologia 5: 62. 1913.

Pileus 12 cm. or less in diameter, single or confluent, irregular, channeled, usually depressed at the center, tomentose, subzonate, with a broad, whitish marginal zone and brownish toward the center, composed of a soft, felty upper layer and a tough, compact lower layer; odor when fresh very disagreeable; taste slight; stipe 1–4 cm. long, 0.5–2 cm. in diameter, uneven, varying greatly in size, composed of a soft, spongy surface layer and a hard, woody axial region, color of the older portions of the cap; spines 2–4 mm. in length, 4–6 per mm., crowded, subulate, terete, white when fresh, becoming light gray to grayish brown when dry; hyphae 3.5–6  $\mu$  in diameter, firm-walled, faintly colored under the microscope, without clamp connections, with slightly thicker walls in the compact regions; basidia 20–30  $\times$  4–5  $\mu$ ; spores 3.5–4.5  $\mu$  in diameter, spherical, coarsely echinulate, subhyaline or faintly colored.

Specimens of *Phellodon vellereus* determined by Banker and *P. amicus* and *Hydnnum amicum* by Banker and Patouillard at The New York Botanical Garden are quite like our Iowa specimen. *Hydnnum putidum* is similar if not identical.

Rare in Iowa. On the ground in October. Reported from Washington and from many states in the eastern United States.

2. CALODON ZONATUM (Fries) Quél. in Cooke & Quél. Clav. Hy-  
     men. 197. 1878. (FIG. 15)

*Hydnnum cyathiforme* Bull. ex Fries, Syst. Myc. 1: 405, in part.  
     1821.

*Hydnnum concrescens* Pers. Myc. Eu. 2: 164, in part. 1825.

*Hydnnum zonatum* Batsch ex Fries, Epicr. 509. 1838.

*Hydnellum zonatum* (Fries) Karst. Medd. Soc. Faun. Fl. Fenn. 5: 27. 1879.

*Phaeodon zonatus* (Fries) Schröt. Krypt.-Fl. Schles. 3<sup>1</sup>: 458. 1888.

*Hydnellum concrescens* (Pers.) Banker, Mem. Torrey Club 12: 157. 1906.

Pileus 1–7.5 cm. in diameter, with a central stipe, gregarious, often confluent, depressed in the center, sometimes subinfundibular, distinctly zoned, with radiating ridges or occasionally rugged, pitted and less zoned, tough, fibrous, usually light colored at the margin to pecan brown or vandyke brown at the center; stipe short, 0.5–2 cm. in length, 0.5–2 cm. in width above, wider and with a spongy, bulbous base below, color similar to the pileus; teeth slender, terete, 1–3 mm. or less in length, 5–6 per mm., vandyke brown; hyphae 2.5–6.5  $\mu$  in diameter, colored under the microscope, without clamp connections; basidia 20–35  $\times$  5–6  $\mu$ ; spores 4.5–6  $\times$  3.5–4.5  $\mu$ , subspherical, coarsely tuberculate, russet brown in mass.

*Calodon zonatum* seems to be closely allied to *C. ferrugineum* and *C. scrobiculatum*. Banker (1913) regards the latter two names as synonyms. Since these are maintained as distinct by more recent writers, as Rea (1921), Bourdot and Galzin (1927) and other European workers, it seems best to keep them separate for the present.

Fairly common in Iowa; on the ground from August to November. Widely reported from the United States.

### 3. CALODON FERRUGINEUM (Fries) Quél. in Cooke & Quél. Clav. Hymen. 196. 1878.

*Hydnnum ferrugineum* Fries, Syst. Myc. 1: 403, *non* Persoon. 1821.

*Hydnellum ferrugineum* (Fries) Karst. Medd. Soc. Faun. Fl. Fenn. 5: 27. 1879.

*Phaeodon ferrugineus* Schröt. Krypt.-Fl. Schles. 3<sup>1</sup>: 459. 1888.

*Hydnellum sanguinarium* Banker, Mem. Torrey Club 12: 152. 1906.

Generally distinguished from *C. zonatum* by the thicker and less zonate pileus, the whitish margin, the mass of tubercles in the middle and the reddish liquid. The two forms are similar and differ little in the microscopic characters. The flesh pink

or onion-skin pink margin of *C. zonatum* described by Coker suggests the pale margin of *C. ferrugineum*. After years of study and observation of fleshy hydnoms, Banker apparently came to regard the red juice as having little weight in the separation of species.

Collected on rich humus in woods. September. Uncommon in Iowa. Widely reported from the eastern United States.

4. CALODON SCROBICULATUM (Fries) Quél. in Cooke & Quél. Clav. Hymen. 197. 1878.

*Hydnum cyathiforme* Bull. ex Fries, Syst. Myc. 1: 405, in part. 1821.

*Hydnum scrobiculatum* Fries, Epicr. 509. 1838.

*Hydnellum scrobiculatum* (Fries) Karst. Medd. Soc. Faun. Fl. Fenn. 5: 27. 1879.

Generally separated from *C. zonatum* by the more rigid, thicker, less zonate and rougher pileus. Bourdot and Galzin indicate that the fungus may be separated from *C. ferrugineum* by the smoother surface and the absence of a purplish liquid. Banker, however, regards *C. ferrugineum* and *C. scrobiculatum* as representing the same species. It seems equally plausible that *C. scrobiculatum* and *C. zonatum* represent one species. Many specimens under both names were studied at The New York Botanical Garden. They clearly merge and are practically indistinguishable. Careful study of living material under natural conditions and in culture if possible seems highly desirable in order to determine satisfactorily the validity of these similar species.

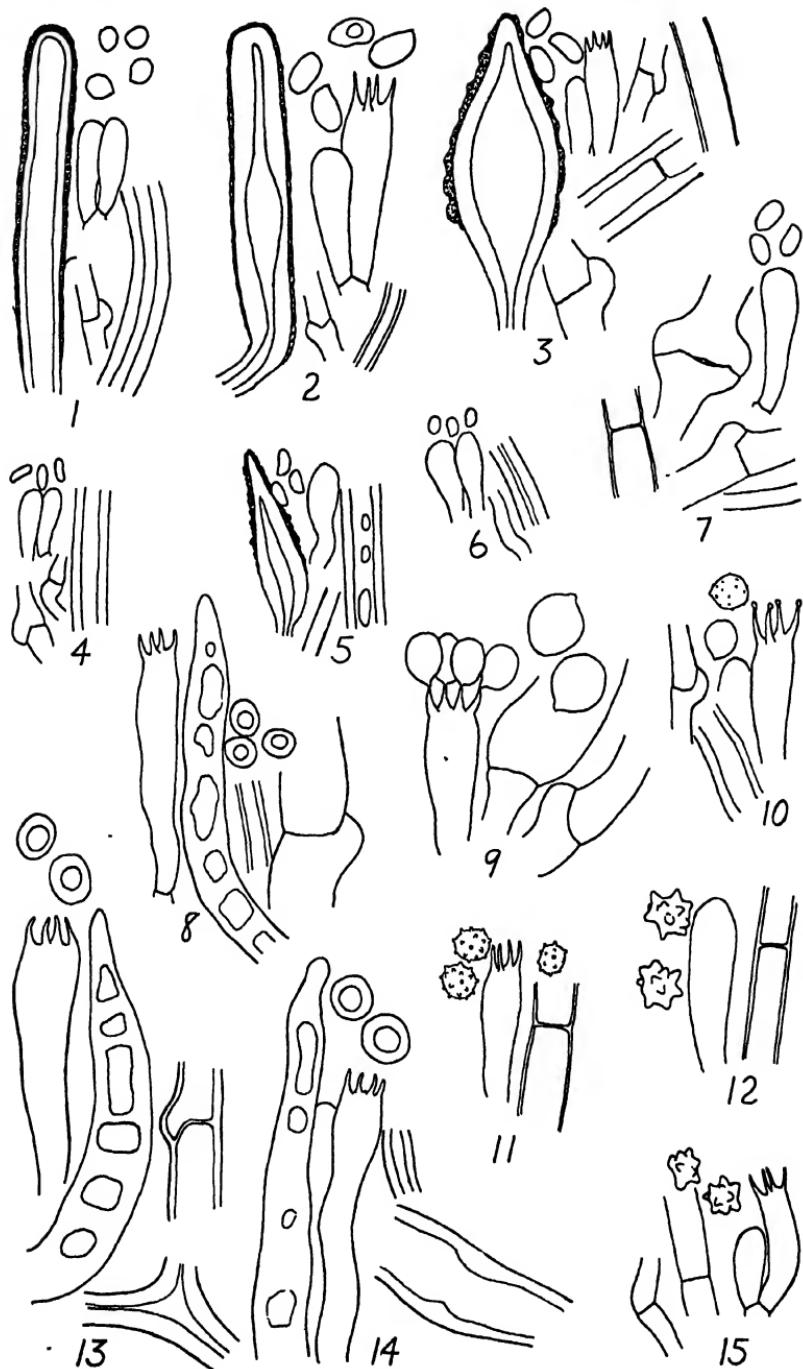
5. CALODON VELUTINUM (Fries) Quél. in Cooke & Quél. Clav. Hymen. 197. 1878. (FIG. 12)

*Hydnum velutinum* Fries, Syst. Myc. 1: 404. 1821.

*Hydnellum velutinum* (Fries) Karst. Medd. Soc. Faun. Fl. Fenn. 5: 27. 1879.

*Hydnum spongiosipes* Peck, Ann. Rep. N. Y. State Mus. 50: 111. 1897.

Pileus 2-12 cm. in diameter, with a central stipe, convex, plane or occasionally depressed at the center, not zoned, finely tomentose, filled with a colorless or reddish liquid, with a soft felty





upper layer and a hard compact lower layer, uniformly cinnamon brown; stipe 1–6 cm. long, 0.8–1.5 cm. in diameter above, becoming wider toward the base, with a compact, hard central core surrounded by a soft felty layer, color of the pileus; spines 1–6 mm. in length, 2–4 per mm., subulate, terete, acute, color of the pileus; hyphae 3–6  $\mu$  in diameter, fairly thick-walled, without clamp connections, seldom over 4.5  $\mu$  in diameter in the spines, brownish in color; basidia 24–45  $\times$  5–8  $\mu$ ; spores 5–6.5  $\mu$  in diameter, spherical, coarsely tuberculate, brown.

Collected in August and September on the ground; uncommon in Iowa. Its occurrence is widely reported throughout the United States.

DEPARTMENT OF BOTANY,  
STATE UNIVERSITY OF IOWA,  
IOWA CITY, IOWA

#### EXPLANATION OF PLATE 33

All figures are drawn with camera lucida at a magnification of 1650 diameters, reduced to  $\times 1000$  in reproduction.

Hyphae, basidia and spores are shown in each figure. Cystidia are included also in figures 1, 2, 3 and 5, and gloeocystidia in 8, 13 and 14.

Fig. 1, *Steccherinum ochraceum*; 2, *S. setulosum*; 3, *S. septentrionale*; 4, *S. adustum*; 5, *S. rawakense*; 6, *S. pusillum*; 7, *S. pulcherrimum*; 8, *Hericium laciniatum*; 9, *Dentinum repandum*; 10, *Auriscalpium vulgare*; 11, *Calodon amicum*; 12, *C. velutinum*; 13, *Hericium Erinaceus*; 14, *H. coralloides*; 15, *Calodon zonatum*.

## NEW AND RARE MYCETOZOA FROM LONG ISLAND

ROBERT HAGELSTEIN

(WITH PLATE 34)

In studying extensive collections of Mycetozoa made on Long Island in recent years, I have come across a form of *Comatricha* which I propose as a new species.

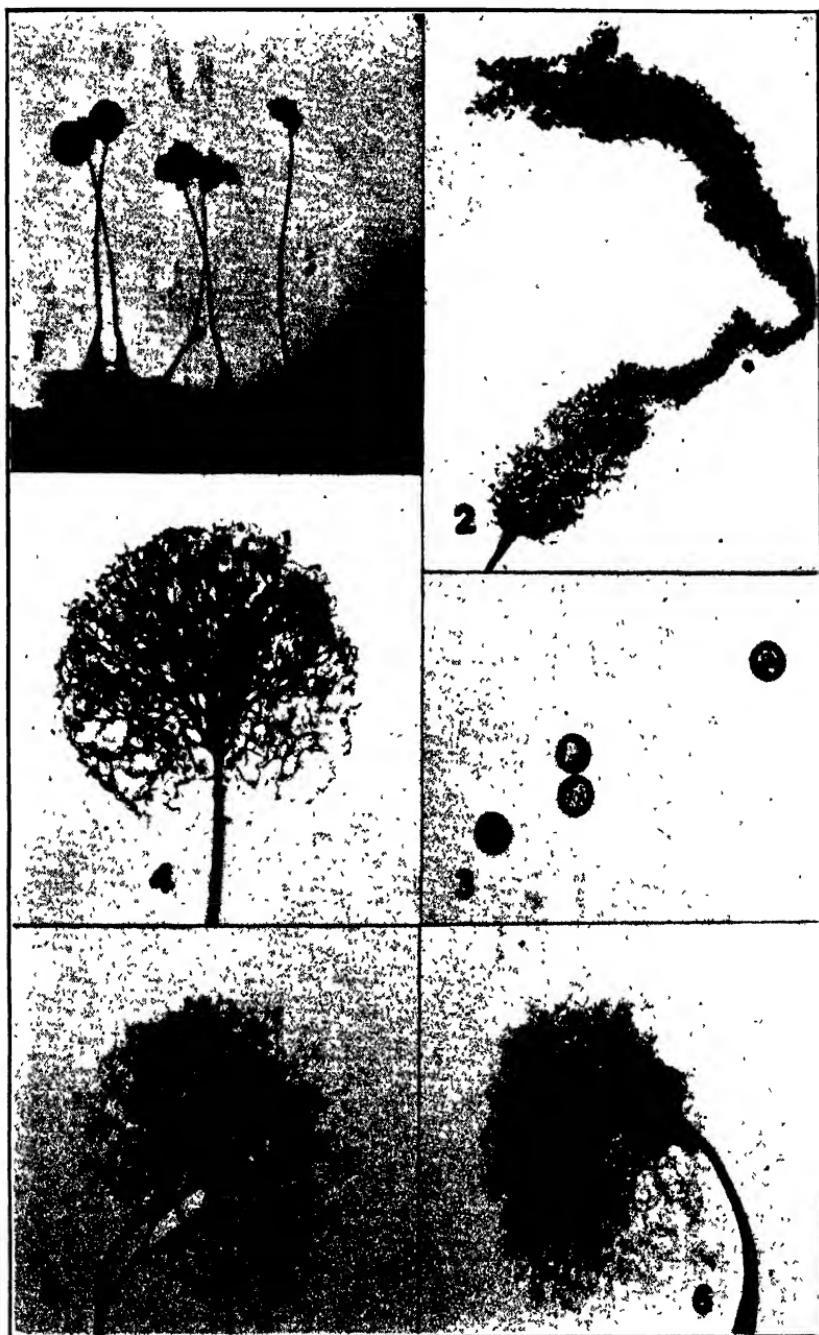
### **Comatricha extendens** sp. nov.

Sporangiis gregariis, globosis, fuscis, stipitatis, ad 4 mm. altis; stipite subulato-gracile, atro, nitente; columella nulla; capillitio rete intricatum formante, elastice protrudente; sporis violaceo-brunneis, minutissime echinulatis, 8.5 - 10  $\mu$  diam.

Plasmodium? Total height 2 to 4 mm., unexpanded. Sporangia globose, stalked, gregarious, 0.4 to 0.65 mm. diam., dark purplish-brown; sporangial wall firm, finally evanescent. Stalk subulate, slender, black, shining, from 4 to 6 times the size of the sporangial body, rising from a distinct hypothallus; the stalk either expanded at the top or splitting into several parts, as many as eight, which form the primary branches of the capillitium. Columella lacking or obsolete. Capillitium a tangled mass of anastomosing purplish-brown threads springing directly from the stalk at the base of the sporangium; threads stouter at the base, becoming uniformly more slender, branched, somewhat looped or netted, but without a surface net; capillitium more or less elastic, ultimately expanding into a cylindrical plume 2 or 3 times the size of the sporangial body. Spores brownish-violet, minutely but distinctly spinulose, 8.5 to 10  $\mu$  diam.

Habitat. Indoors, on the lower side of flooring in close proximity to steam pipes; in a moist environment with temperature 90 to 100 degrees Fahr. Mitchell Field, Long Island, N. Y., March 1930.

The development is perfectly matured and uniform throughout, and does not appear to be abnormal. Except for the lacking columella, replaced by an extending capillitium attached directly to the top of the stalk, the form is like *Comatricha nigra* (Pers.)



COMATRICA SP.



Schröt. The absent columella would indicate a genus separated from *Comatricha* as the columella is an important feature of the latter. I am convinced, however, that *C. extendens* has developed from *C. nigra* through *Comatricha elegans* (Racib.) List., and have specimens of *C. elegans* from Mitchell Field, where, instead of continuing into the sporangium as a columella, the stalk divides into two or more branches below the sporangial base, with the capillitium retaining its globose shape. These forms, in my opinion, are intermediate between *C. elegans* and *C. extendens*, but nearer *C. elegans*. From the same locality, a series of intermediate forms connect *C. elegans* with *C. nigra*.

The warm, moist environment of the habitat suggests that the species may be looked for in the tropics. Mitchell Field is an army aviation post, and in the past, large quantities of tropical woods have been brought there for various purposes.

It is noteworthy to record also the collection of *Comatricha lurida* List. which, so far as I know, has not been reported from North America. The species was found twice, on leaves, at the Albertson kettle hole, Long Island, in July 1927. The specimens have globose, light brown sporangia, on short slender stalks. The columella extends about half way into the sporangium, and divides at the top into the primary branches of the capillitium. While some of the threads are bent downwards, there are no attachments to the lower part of the columella, nor is there any firm, peridial base, with attached threads, as in *Comatricha rubens* List., a closely allied species. The spores are light violet-gray in color, irregularly globose, distinctly warted, and measure about 7  $\mu$ .

MINEOLA, NEW YORK

#### EXPLANATION OF PLATE 34

Fig. 1-3, *Comatricha extendens*: 1, group of sporangia,  $\times 10$ ; 2, top of stalk and capillitium,  $\times 50$ ; 3, spores,  $\times 500$ ; 4-5, *Comatricha elegans* (Racib.) List. from Mitchell Field; 4, capillitium,  $\times 100$ ; 5, intermediate form approaching *Comatricha extendens*, capillitium,  $\times 100$ ; 6, *Comatricha lurida* List. from Long Island, capillitium,  $\times 100$ .

# A NEW SAPROPHYTIC SPECIES OF LAGENIDIUM, WITH NOTES ON OTHER FORMS

JOHN N. COUCH

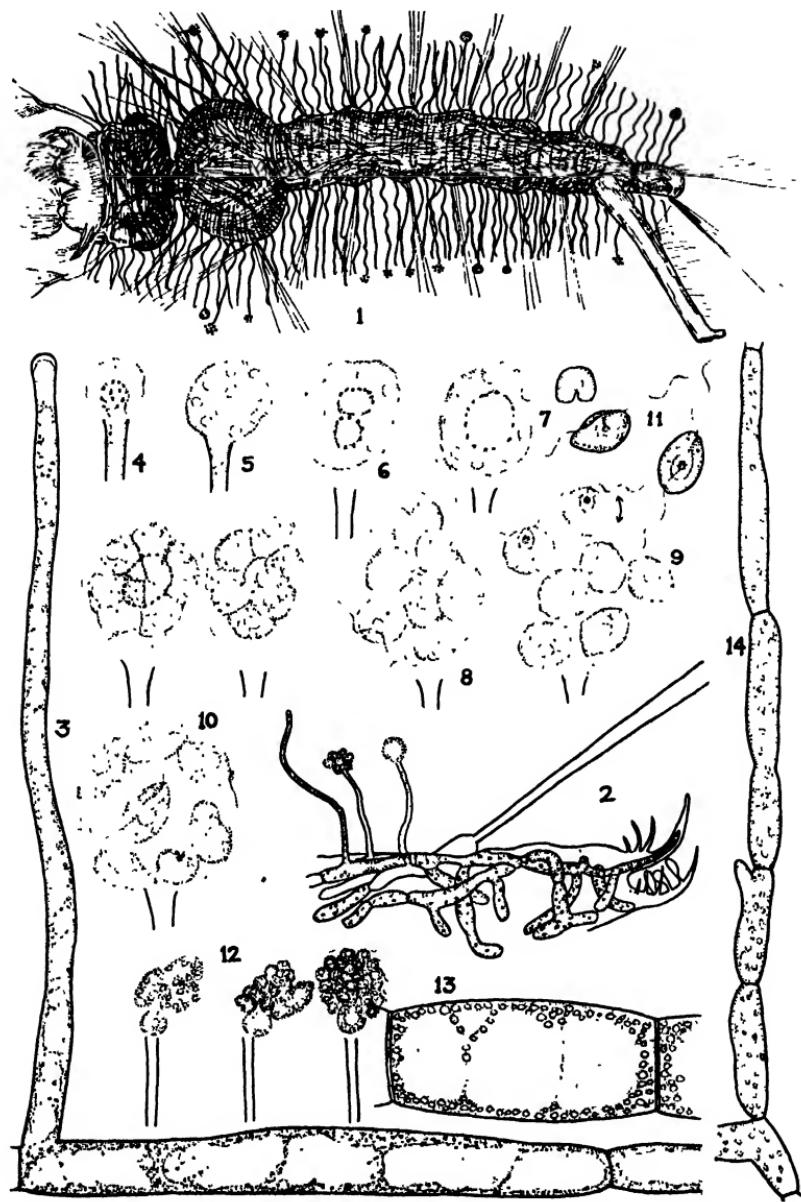
(WITH 40 TEXT FIGURES)

Up to the present time so far as known no species of the Aencylistales has been grown in pure culture either on solid or in liquid media. The new species described below, while a facultative parasite on mosquito larvae, *Daphne* and copepods, can be cultured on a variety of nutrient agars. It is also noteworthy because it probably represents a connecting link between these two lower orders of non-filamentous fungi and the filamentous Phycomycetes.

To date about fifteen species of *Lagenidium* have been described, only three of which have been reported in the United States and these from the northeastern states. In the present paper, in addition to the new species, four species known heretofore only from Europe are reported in America, as well as another form, the specific identity of which is doubtful.

## *Lagenidium giganteum* sp. nov. (FIGS. 1-19)

The main hyphae are segmented, being constricted or not constricted at the septum, the segments sometimes separating from each other; branched. When growing on a copepod, *Daphne*, or mosquito larva, the large segmented hyphae are within the host, but numerous delicate hyphae extend from the host for a distance of one or two millimeters to form a fringe which has much the appearance of a delicate species of *Aphanomyces*. Hyphae 6-40  $\mu$  thick, the segments 50-300  $\mu$  long. The hyphal walls contain cellulose giving a purplish reaction with chlor-iodide of zinc. The protoplasm has the pale whitish gleam as in the Aencylistales. Any segment may become a sporangium. The sporangium empties its content in an undifferentiated, naked mass (or sometimes several masses) through a tube, the dimensions of which are 6-10  $\times$  50-300  $\mu$ . This mass becomes differentiated into a variable



FIGS. 1-14. *Lagenidium giganteum*: 1, mosquito larva parasitized by *Lagenidium* (note large, internal, segmented hyphae and delicate external threads most of which are long emergence tubes for sporangia),  $\times$  about 14; 2, post-abdominal region of *Daphne* showing fungus within,  $\times$  about 135; 3, sporangium with emergence tube,  $\times$  625; 4-10, discharge of sporangial contents and formation of spores,  $\times$  about 570; 11, spores in motile state,  $\times$  about 570; 12, sporangium from which contents were discharged in three separate lumps,  $\times$  about 230; 13, hyphal segment with large vacuoles,  $\times$  about 625; 14, sketch of part of a hypha,  $\times$  325.

number of laterally biciliate zoospores. Zoospores  $8-9 \times 9-10 \mu$ , their movement as in *Achlya* but rather sluggish. Monoplanetic. Sexual reproduction not observed.

Weakly parasitic on mosquito larvae, copepods and *Daphne*. Also culturable as a saprophyte. This fungus first appeared on *Daphne* and copepods in material collected from the lake at Mountain Lake, Va., during the summer of 1933. Its remarkable characters were noted at the time and some drawings and notes were made. A few weeks later the same fungus was again found on mosquito larvae at Chapel Hill, N. C., and this time was isolated in pure culture. It appears to be more closely related to *Lagenidium* than to any other known fungus, and due to its unusually large size I am describing it as *L. giganteum*.

The fungus was isolated in pure culture by picking up with a platinum loop a drop of water containing some of the discharged zoospores and smearing this drop over a corn meal agar plate. After the spores had germinated, several of the germlings were cut out under the binocular microscope and planted separately on fresh, corn meal agar plates.

Growth proved to be exceedingly slow and so attempts were made to find a better culture medium. On corn meal agar the culture attained a diameter of 0.7 cm. in 5 days, the threads being rather slender and considerably coiled. On corn meal agar slightly alkaline, about pH 7.5, the growth was about 1 cm. in diameter after 5 days, the threads being rather thick. No growth was obtained when the alkalinity was increased beyond pH 7.5 nor when the culture medium was acidulated with phosphoric acid. In 1 per cent peptone growth very poor; in 1 per cent beef extract (Difco) growth excellent, about 2 mm. diameter after 3 days, hyphae of uniform diameter, not constricted at septa. In equal parts of 1 per cent peptone, 1 per cent maltose, 1 per cent meat extract, growth fair, about 1.5 mm. diameter after 3 days, hyphae with numerous swellings. On maltose peptone agar (2 per cent agar, 0.3 per cent maltose, 0.1 per cent meat peptone) the threads were straight, the hyphae being constricted at the segments. On corn meal agar the hyphae were coiled but not constricted at the segments.

Soon after the fungus was isolated in pure culture on maltose

peptone agar a sector of growth appeared that was easily recognizable with the unaided eye as being distinct. The new type of growth was composed of threads 8–20  $\mu$  thick, mostly 12  $\mu$  thick. The threads branched irregularly, contained few septations, and formed an open type of growth. On the same medium the old type of growth was composed of compactly arranged, much septate, rarely branched, straight threads. The two types of growth have been cultured separately for several months, both retaining their individual growth characters as shown in the photographs (FIG. 15, 16).



FIGS. 15 AND 16. *L. giganteum*. Two strains growing under the same conditions on maltose-peptone agar, original strain on right, mutant on left,  $\times$  about 2½.

Each segment may form a sporangium. A long emergence tube grows out from each segment, the contents of which, however, show no differentiation into spore origins. The wall at the tip of the emergence tube thickens and softens, finally giving way to the pressure within. First there emerges a globose mass of hyaline material and the granular protoplasm flows out into this. Apparently the very delicate vesicle which later can be seen to surround the developing spores is derived from this substance. This mass of protoplasm is globose or more usually subglobose and contains numerous, small, contractile vacuoles. At this stage the protoplasm may show slight twitching movements. Suddenly one or two vacuoles appear in the center of the mass. These grow rapidly in size, fusing, if more than one is present, to

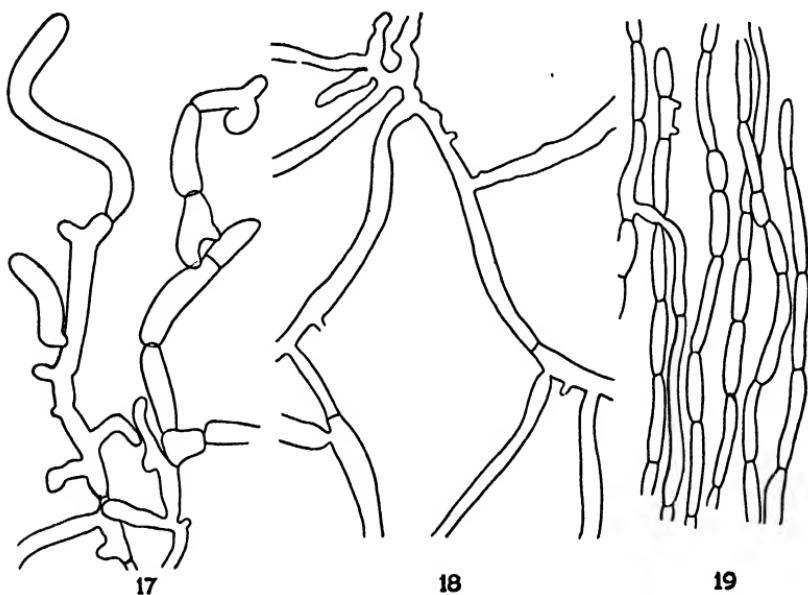
form the conspicuous central vacuole. This central vacuole is lined with small but conspicuous granules. The spore origins now become evident, each with a small distinct vacuole just opposite which two cilia stubs appear in the cytoplasmic membrane. Simultaneously cleavage furrows appear, extending outwards from the large central vacuole. The whole mass at this stage rocks and jerks feebly as a result of the motion of the young cilia. Suddenly the central vacuole disappears, the spore mass shrinking, and the spores sticking together motionless for several minutes. The spores then begin rounding off, separate from each other, and about 3–5 minutes after the clumping stage, begin rocking and jerking again. Within 8 to 10 minutes more the spores change from irregular rounded masses to the typical "kidney-bean" shaped spores. A few seconds after the spores have reached maturity the delicate vesicle is broken and the spores swim away. In most cases I have been unable to see a vesicle.

Frequently the contents of the sporangium may emerge and form not one spherical mass of protoplasm but several masses, in which event the development of the zoospores will be progressive, the mass of protoplasm which emerged first forming its spores first, etc.

No detailed studies on the development of the sporangia of *Lagenidium* have yet been published but from my observations on *L. Oedogonii* it appears that development in that species is similar to that described here. The noteworthy feature is the large central vacuole in the discharged mass of protoplasm which seems to play the same rôle here as in the sporangium of *Saprolegnia*. In *Pythium*, however, there is no large vacuole in the discharged mass of protoplasm.

*Lagenidium giganteum* occupies a unique position in the Acyclistales. All the other species so far described are obligate parasites, so far as known, and have a rudimentary mycelium. The present species would seem to be intermediate between the filamentous Phycomycetes and the non-filamentous forms. It may be cultured on a variety of media, on some of which an extensive mycelium is developed. In spite of the wide discrepancies between this species and the other members of the genus *Lagenidium* it would seem best to place this species in *Lagenidium*, at least until the sexual stages can be found.

A few experiments have been carried out to see if this fungus would kill healthy mosquito larvae. Two large, healthy larvae were placed in Petri dish containing spring water, and *Lagenidium* in spore-forming state was added Dec. 13, 10:30 A.M. On Dec. 14 one of the larvae was seen eating the fungus. On Dec. 16 both larvae were alive. On Dec. 17, 2 P.M., one larva was quiet

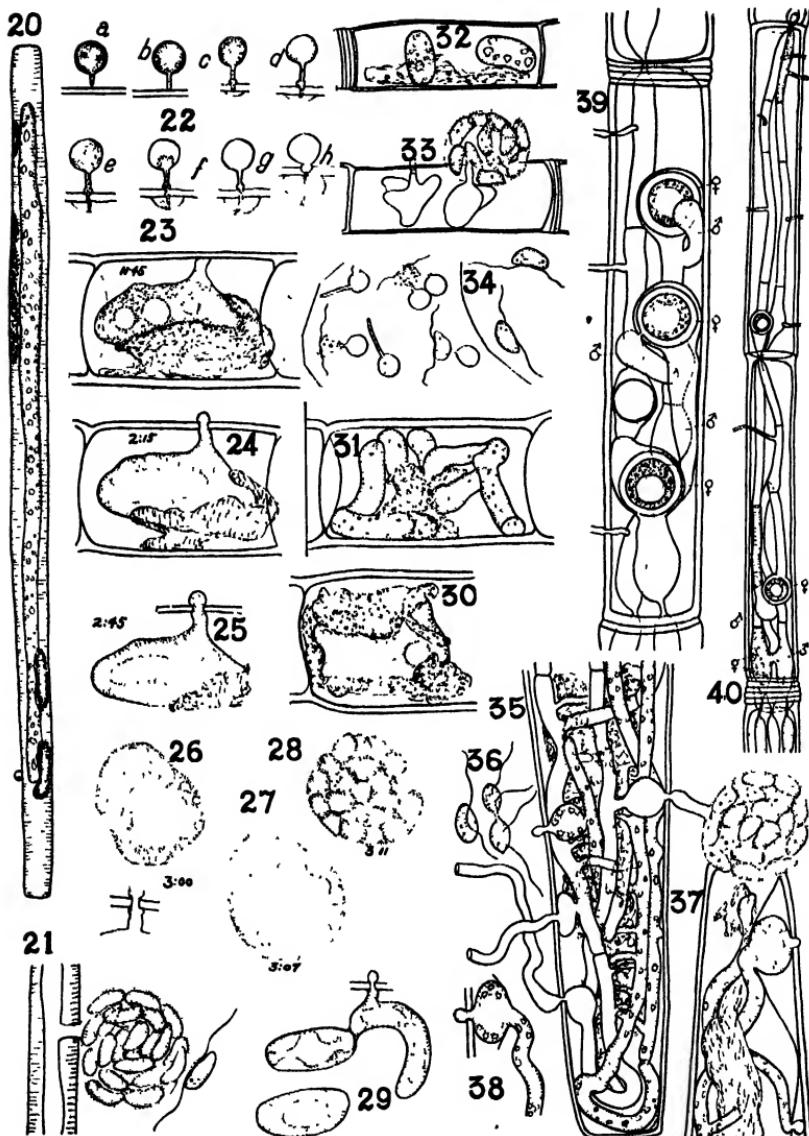


FIGS. 17-19. *L. giganteum*. 17, sketch of hyphae on corn meal agar; one of earliest cultures,  $\times 140$ ; 18, sketch of threads from mutant, strain from fig. 15 (note open irregular growth, few septations and thicker hyphae); on maltose-peptone agar,  $\times 110$ ; 19, sketch of hyphae from original strain (note compact, regular growth and abundant regular septations); on maltose-peptone agar,  $\times 110$ .

(almost dead), infected in region of eyes, other infected near tuft of hairs on head. Essentially same results obtained in another experiment in which 7 larvae were used. Whether the fungus killed the larvae or whether the larvae become weakened and were then parasitized, I cannot yet say.

#### LAGENIDIUM CLOSTERII de Wildeman. (FIGS. 35-38)

Thallus composed of long, narrow, straight or much twisted, branched threads. Threads sometimes of more or less uniform diameter throughout (about  $1.8\text{--}2.8 \mu$  thick), sometimes swollen



FIGS. 20 AND 21. *Lagenidium brachystomum* Scherffel. 20, elongated, worm-like, non-septate thallus in cell of *Synedra* sp., drawn 11:30 A.M.; 21, spore formation, 6 P.M., in same thallus, spores surrounded by bladder; on right, a biciliate zoospore, both figures  $\times$  about 400.

FIGS. 22-31. *Lagenidium Oedogonioides* Scherffel. 22 a-g, stages in the germination and penetration of a zoospore, the process occupying about an hour and a half, slightly diagrammatic (note appressorium and callus on inside of host wall); h, a spore, with appressorium, whose germ tube has

and of quite irregular diameter. Hyphal contents of characteristic appearance with numerous, large, shiny, irregular bodies, and with pale cytoplasm. Thallus when mature breaking up into units, each of which may form a sporangium. From the sporangium arises a short branch which enlarges at the end to form a spherical body. From this body there arises a fine thread which penetrates through the wall of the *Closterium* and may grow out for some distance ( $20-30 \mu$ ) away from the host. The sporangium discharges its contents into a vesicle in which zoospores are differentiated. Zoospores biciliate, about  $3.8 \times 5.6-6.3 \mu$  in the motile state, shaped as in *Pythium*, with several shiny globules. The spores swim with a peculiar, hopping motion, sometimes however they swim in a smooth, regular, spiral path, as is characteristic of the zoospores of *Achlya*, *Pythium*, etc. Sexual reproduction not observed.

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successfully penetrated host wall (note large callus),  $\times 675$ ; 23-28, stages in late development of sporangium: 23, note granular cytoplasm, vacuoles and hyaline, emergence tube flattened out against host wall; 24, large, central vacuole and tube penetrated through host wall; 25, note central vacuole extending out into tube; 26, content of sporangium discharged (note large vacuole and numerous smaller ones); 27 and 28, stages in spore formation; in fig. 28 there are very faint signs of a bladder; about seven minutes after spores became distinct, bladder broke and spores dispersed; 29, above, a sporangium in which spore origins (?) have appeared and below five minutes later the origins (?) have disappeared, leaving large central vacuole with a smooth outline; 30 and 31, sporangia both of which emptied a few hours after sketches were made; figs. 23-31  $\times$  about 480.

Figs. 32-34. *Lagenidium* sp.: 32, two young sporangia in *Oedogonium* cell; 33, two sporangia, one of which has just discharged its content which has now become organized into spores (note bladder); 34, two spores on right in motile condition just after bladder broke; on left a group of spores kept under observation for about three hours, some spores germinating by delicate tube, others emerging from cysts (note delicate bladder around spores which have just emerged from cysts); one spore is shown which had begun to germinate but which reversed its action and is emerging from cyst, all  $\times$  about 625.

Figs. 35-38. *Lagenidium Closterii* de Wildeman: 35, part of a *Closterium* cell nearly filled with parasites (note striking resemblance between young threads and *Pythium*); on right discharged content of sporangium is being differentiated into spores within a bladder; 36, three spores, two of which are joined by a narrow band of cytoplasm; 37 and 38, formation of exit tube of sporangium, all  $\times$  about 560.

Figs. 39 AND 40. *Lagenidium Marchalianum* de Wildeman: 39, several oögonia and antheridia within *Oedogonium*,  $\times$  about 625; 40, long threads of parasite, young and empty sporangia, and oögonia and antheridia and one parthenogenetic egg,  $\times$  about 335.

Collected only once (No. 6, April 23, 1933) in roadside ditch near Wilmington, N. C., on *Closterium* sp. Cell membrane containing cellulose and giving a lavender color with chlor-zinc-iodide. Found once in Bohemia by Karel Cejp (Bull. International de l'Académie des Sciences de Bohême *pl. 1 & 2.* 1933).

The elaborately developed mycelium of this parasite in the early stages before it breaks up into sporangia, may easily be confused with that of certain species of *Pythium* parasitic on algae. The large shiny granules and the clear cytoplasm easily distinguish the thallus from that of *Pythium*.

**LAGENIDIUM MARCIALIANUM** de Wildeman. (FIGS. 39, 40)

Thallus composed of elongated, segmented, sometimes branched threads which may extend throughout the length of several cells of *Oedogonium* sp. Threads 2.2–6.7  $\mu$  thick, slightly or not at all constricted at the segments, or sometimes swollen at the segments, more or less straight, but often irregular, sometimes confined to one cell; threads narrowly constricted when passing through the cross walls of the host, about 1  $\mu$  thick. Segments 30–60  $\mu$  long. Each segment may become a sporangium, giving rise to a very delicate (1.5–2  $\mu$  thick), short exit tube which pierces the host wall, usually extending out only 4–5  $\mu$  beyond the host wall. Spore discharge not observed. Oögonia and antheridia abundant. Oögonia usually intercalary, rarely terminal, formed by the enlargement of one of the segments, up to 20  $\mu$  thick; eggs 8–14  $\mu$ , with very thick, smooth walls; antheridia may arise by the enlargement of the cell adjacent to the oögonium, this cell being directly transformed into an antheridium or the cell adjacent to the oögonium may branch to form the antheridium, or the antheridium may arise from the cell of another thread. Only one antheridium to an oögonium and sometimes the egg may be formed parthenogenetically. Antheridium emptying its entire contents through a small tube into the developing egg.

Collected only once on a large species of *Oedogonium*, July 1933, in the lake at Mountain Lake, Virginia. This species shows a close relationship to *L. Rabenhorsii* in the appearance of the mycelium, sporangia, oögonia and antheridia. However, that species, with one exception (Graff, Mycologia 20: 169, 1928, who

reports it on *Oedogonium plusiosporum* Wittr.), has been reported only on filamentous forms of the Conjugales, the mycelium is limited to one cell, and the eggs are from 15–20  $\mu$  thick, considerably larger than in the present species.

Of the several species described on *Oedogonium*, *L. Oedogonii* Scherffel, *L. Zoppii* de Wildeman, *L. syncytiorum* Klebahn, *L. Marchalianum* de Wildeman, it seems, from a comparison with the original descriptions and figures, that the present species is closest to *L. Marchalianum*. That species has a mycelium the threads of which are about 4–5  $\mu$  thick and composed of more or less cylindrical segments. The threads are branched and extend through as many as six or seven host cells. Sexual reproduction not observed. The present species differs from *L. Zoppii* in having a smooth egg membrane while in *L. Zoppii* the membrane is minutely rough. It may be that these three species are the same but the evidence at hand at present does not justify such a conclusion.

#### LAGENIDIUM BRACHIYSTOMUM Scherffel. FIGS. 20, 21)

Thallus composed of a long, unbranched, non-septate, delicately walled tube, 4–7.5  $\times$  150–250  $\mu$ , with whitish gleaming protoplasm and shiny fat (?) globules. The entire tube becomes one sporangium which empties its contents through a short neck. Spores formed within a vesicle, laterally biciliate, 4  $\times$  6  $\mu$  when swimming. Sexual reproduction not observed.

Collected only once in *Synedra* sp., Feb. 18, 1932, Chapel Hill, N. C.

Of the three species of *Lagenidium* (*L. Cyclotellae* Scherffel, *L. enecans* Zopf, and *L. brachystomum* Scherffel) which have been described on diatoms the present species seems to agree best with *L. brachystomum* in having a simple, unbranched, non-septate thallus, and a short emergence papilla.

#### LAGENIDIUM sp.? (FIGS. 32–34)

In the same material in which the *Lagenidium brachystomum* appeared there was a very small parasite on *Oedogonium* which at the time I took to be the same as *L. brachystomum* on *Synedra* sp. Without infection experiments, however, the identity cannot be

determined with certainty. Thallus ovoid or somewhat irregular, with the whitish protoplasm and glistening fat bodies characteristic of the genus,  $7-10 \times 15-20 \mu$ , non-septate. Spores surrounded by a vesicle the presence of which, though faint, could be attested by the fact that a spore came to rest on the vesicle before it broke. Spores laterally biciliate, about  $4 \times 7.5 \mu$  in the motile condition, about  $5 \mu$  thick when encysted. Spores diplanetic, coming to rest after swimming for a short while, encysting and then emerging from cysts 1 to 3 hours later, to swim again in the laterally biciliate condition. Even the single spores which emerged from the cysts appeared to be surrounded by a delicate vesicle. In a few cases encysted spores which had begun to germinate emerged from the cysts leaving both germ tube and cyst empty. Sexual reproduction not seen.

Collected only once, February 18, 1932, Chapel Hill, N. C.

**LAGENIDIUM OEDOGONII Scherffel. (FIGS. 22-31)**

Sporangia within the cells of *Oedogonium*, usually one sporangium to a cell, rarely two. Irregularly ovoid, sometimes lobed, very rarely filamentous,  $20-25 \times 35-52 \mu$ , the filamentous forms up to several hundred microns long and composed of a coiled thread. Protoplasm with a pale whitish gleam and with conspicuous shiny globules. Sporangium when just mature with numerous, small vacuoles; spore origins delimited within sporangium but quickly disappear, leaving a large, central vacuole. Exit tube short, extending through the host wall, and only a few microns beyond. Contents of sporangium emerging to form an irregularly globoid mass surrounded by a very indistinct vesicle; with a large central vacuole and numerous smaller ones. Spores laterally biciliate, pointed at the anterior and rounded at the posterior end. Spores swimming smoothly as in *Achlya*, rotating on their long axis and at the same time moving in a spiral path. Encysted spores about  $6.6 \mu$  thick.

This species, first described by Scherffel in Hungary, has been collected by me near St. Louis, Mo., on *Oedogonium*. The material from Missouri agrees in most details with Scherffel's except that the spores in his species were not surrounded by a bladder; also he found sexual reproduction, whereas I found none. Scherff-

fel describes the spores as being sometimes diplanetic. In such cases the spores are formed within the sporangium, emerging individually to collect in an irregular, spherical cluster at the tip of the emergence tube. Here they encyst, remain for some time, and later emerge from the cysts to swim in the laterally biciliate condition. Such spores are thus diplanetic as in *Achlya*. This species is very distinct from other species of *Lagenidium*, being characterized by the irregularly ovoid, non-segmented thallus.

#### SUMMARY

A new species of *Lagenidium* is described as *L. giganteum*. It is a facultative parasite on mosquito larvae, *Daphne*, and copepods. The development of the sporangia is described. Sexual reproduction was not observed. It is also saprophytic, being culturable on a variety of nutrient agars. Soon after the fungus was isolated on agar a mutation occurred. The mutant can be recognized by the more irregular growth, thicker hyphae, and fewer septations. Both the mutant and the original strain have been kept in culture for over a year. This fungus probably represents a connecting link between the non-filamentous Phycomycetes and the filamentous forms. *Lagenidium Closterii*, *L. Marchalianum*, *L. Oedogonii* and *L. brachystomum* are described from America for the first time. A doubtful species of *Lagenidium* also is described.

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# A COMPARATIVE STUDY OF CERTAIN SPECIES OF MARASMIUS AND COLLYBIA IN CULTURE<sup>1</sup>

JEAN D. ARNOLD

(WITH PLATES 35 AND 36)

## INTRODUCTION

During recent years considerable attention has been devoted to the subject of sexuality in the Hymenomycetes, with the result that conditions prevailing in many species have been determined. In the Agaricaceae the genus *Coprinus* has received the most study, but species of *Hypholoma*, *Panaeolus*, *Pholiota* and other genera have also been investigated.

It is well known to students of the Agaricaceae that some of the small members of the genus *Collybia* resemble the genus *Marasmius* very closely, not only in size and general appearance, but also in the ability of the dried pileus to revive when moistened. Since little attention has been given to species of *Marasmius* or the marasmoid species of *Collybia* with regard to their development and sexual behavior, several species of *Marasmius* and *Collybia* were used in the present study. The following species received the most attention in this work: *Marasmius elongatipes* Peck, *Collybia tuberosa* Fries, *C. cinnata* Fries and the form hitherto recognized as *C. cinnata* Fries var. *Cookei* Bres. (1).

## HISTORICAL

As early as 1860 there is a record of simple experiments dealing with *Collybia tuberosa*. In "The Gardeners' Chronicle and Agricultural Gazette" for that year there is a mycological note written by Berkeley (3, p. 456). *Collybia tuberosa* is referred to there as *Agaricus tuberosus*, and Berkeley points out that the tubers them-

<sup>1</sup> A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Michigan. March 23, 1934. Papers from the Department of Botany and the Herbarium of the University of Michigan, No. 545.

selves were once considered a distinct genus, and were known as *Acrospermum cornutum*. The last paragraph of the article reads as follows: "It may not be uninteresting to some of our readers to state that many of these tuber-like bodies will produce their true fruit if placed under proper conditions of air, light, heat, and moisture. We have ourselves shown this to be the case in one instance many years since, and Mr. Currey, who bids fair to follow in the steps of the Messrs. Tulasne in the study of Mycology, has been successful in several more or less important cases."

However, little interest was shown in the study of this group of fungi until 1918, when Mlle. Bensaude's thesis (2) was published in Paris. This outstanding piece of work on the life cycle and sexuality of the basidiomycetes aroused widespread interest. The history of subsequent investigations dealing with this general topic has been reviewed so thoroughly elsewhere (9) (14), that mention will be made here of only those studies relating to the species dealt with in this investigation.

Most of the information on hand at present concerning the species here involved is due to the investigations of Kniep (12) (13), who reported heterothallism in *Collybia circata* and *C. tuberosa* and the occurrence of haploid fruit bodies in the latter species.

Kniep (14, p. 413) also studied *C. circata* in more detail and found as the result of mating monosporous mycelia obtained from basidiospores from diploid fruit bodies, that this species was tetrapolar with regard to the segregation of its sexual factors. He also found that monosporous mycelia originating from basidiospores from two separate fruit bodies both collected from the same wooded area, were completely interfertile when paired in all possible combinations. Monosporous mycelia from the spores of each pileus, when paired with other monosporous mycelia of the same pileus, gave a clear case of tetrapolarity.

#### TECHNIQUE AND MEDIA USED

The original cultures obtained in the study of these species were all procured from fresh material, either from spores or from tissue from the pileus or sclerotia when these were present, using the customary precautions against contamination. When mono-

sporous cultures were made, Kauffman's method (11) for the isolation of single spores by spraying on agar plates was used, with the following modification: Each time a plate was sprayed, half the suspension in the capsule was poured out and the volume made up again using sterile distilled water. This process was repeated several times, thereby making a series of dilutions among which a plate was obtained in which the spores were sufficiently scattered so that single spores could be isolated with little difficulty.

For cutting out the spores, a nichrome wire inoculating needle, flattened and pointed so that it resembled a minute scalpel, proved very serviceable. As each spore was cut out it was transferred to a sterile agar plate. After about two weeks' growth, the cultures were examined for clamp connections. If none appeared, transfers were made to malt agar slants. All cultures were stored on shelves in diffuse light at a temperature of about 20° C.

At first Kauffman's nutrient agar as given by Lohman (16) was used in the spore germination tests. Although it was found to be a satisfactory medium, it was later given up in favor of plain malt extract agar, which gave excellent development and was more easily prepared. The following proportions were used in the preparation of the malt agar:

Malt extract.....	25 grams
Agar.....	20 grams
Distilled water to make.....	1 liter

This was sterilized in an autoclave for 25 minutes at 12 pounds pressure. If the malt extract is completely dissolved in the water before being placed in the autoclave, and if a fairly good grade of agar is used, filtering is not necessary for ordinary purposes. Other media used were: Kotila's agar (15), Poole's medium for developing sporophores in *Collybia dryophila* (20), Etter's medium (8) and certain modifications of the last two. The following combinations were found to be the most useful for inducing fruiting in the species of *Marasmius*:

*Medium 5.*

Powdered oak wood.....	40 grams
Malt extract.....	10 grams
Peptone.....	0.1 gram
Water.....	120 cc.

*Medium 10.*

Corn meal . . . . .	50 grams
Agar . . . . .	15 grams
Prune extract . . . . .	1 liter
(75 g. prunes in 1 l. water)	

The media used for the growth and fruiting of the *Collybia* species required different constituents. On Kauffman's and Kotilla's agars their growth was slow and scanty. However, since *Collybia tuberosa* and *C. cirrata* both grow upon decaying fungous material, it seemed logical to incorporate such material into the media used for their culture. In the vicinity of Ann Arbor, Michigan, *C. cirrata* grows in great abundance upon shrivelled remains of *Boletus luteus*. Accordingly, large numbers of these polypores were gathered in the autumn and dried. When needed, the dried *Boletus* caps were cooked in a little water and used as a substratum. This was placed in glass capsules 10 cm. in diameter and 7 cm. deep, and sterilized in an autoclave for 20 minutes at 15 pounds pressure. All three species of *Collybia* fruited well on this medium, producing fruit bodies (PLATE 36, FIGS. 8, 9). A decoction was also made by gently boiling for about an hour some of the dried caps in a quantity of water just sufficient to float them. To each liter of the filtrate 15 grams of agar were added. The mycelia of the three species of *Collybia* grew moderately well on this medium (PLATE 36, FIG. 1). A modification of Kauffman's agar was also used. This included all the nutrient materials as given in Kauffman's formula (11), but *Boletus* extract was used instead of water. The fungi grew fairly readily upon this, but fruiting was reduced (PLATE 36, FIG. 2 AND 3). This medium was used in small Erlenmeyer flasks, and the cultures were grown both in the dark and in diffuse light. Various media using prunes or prune extract were tried, but none of the three species of *Collybia* would grow at all on any medium containing prune. Similar results were experienced by Miss Mounce (17) with *Fomes pinicola*. The medium which was found to be most satisfactory was discovered in an attempt to duplicate as nearly as possible, under aseptic conditions, the natural habitat of the fungi. This was called medium 19 and was prepared as follows:

Ordinary greenhouse soil was placed in the bottom of glass capsules to a depth of 1.5–2 cm. and saturated with water. A dried pileus of *Boletus luteus* (or several, if small) was then laid on top of the soil, and the glass cover placed on the capsule. No cotton was used with the glass cover, as it was found that the fungi thrive better with freer access of air. The capsules and contents were then sterilized in the autoclave at 15 pounds pressure for 30 minutes, and when cool were inoculated. All three species of *Collybia* grew well on this medium and produced fruit bodies generally a little larger than those found in nature. This medium was also used in flasks with fair success (PLATE 36, FIG. 7). It was found that when the soil was sterilized first in a dry oven at a temperature of 140–150° C., and the *Boletus* and water added later and subsequently sterilized in the autoclave as above mentioned, no growth of the fungi occurred.

#### RESULTS

**GERMINATION OF BASIDIOSPORES.** Good spore germination was obtained on Kauffman's nutrient agar and on plain malt extract agar in petri dishes at room temperature (approximately 20–21° C.) in the following species: *Marasmius alliatus* (Schaeff.) Schröt., *M. capillaris* Morg., *M. elongatipes* Peck., *M. epiphyllus* Fries, *Collybia cirrata* Fries, *C. cirrata* Fries var. *Cookei* Bres. and *C. tuberosa* Fries. *Marasmius elongatipes* and the three species of *Collybia* fruited more readily than the other species, with the result that these species were studied in greater detail.

**HAPLOID AND DIPLOID MYCELIA; OIDIA.** In *Marasmius elongatipes* it was somewhat difficult to recognize any macroscopic difference between haploid and diploid mycelia. As a rule, the haploid mycelia were flatter, lying closer to the agar than the diploid mycelia, which were more fluffy. These differences, however, are only relative. There was a decided difference in the rate of growth, the diploid mycelium sometimes growing twice as fast as the haploid under identical external conditions. Different strains of the same species may show pronounced variation. Some of the haploid mycelia of *M. elongatipes* are brown and felty in appearance, and other haploid mycelia in the same species are white and fluffy. The diploid mycelia also may be either

pure white or brown. This will be discussed in greater detail later. No oidia have been observed on either the diploid or the haploid mycelia of *M. elongatipes*.

In each of the three species of *Collybia* studied it is easy to distinguish between haploid and diploid mycelia. All the mycelia are white or slightly creamy in color, but the haploids generally have a more opaque and powdery appearance than the diploids. Sometimes the haploid mycelium lies close to the agar, but at other times it is deep and fluffy, and always very powdery. The diploid mycelium differs in presenting radiating strands of a rather coarse nature, which are very conspicuous. The difference between a typical haploid and a typical diploid mycelium in this group is well shown by plate 36, figure 10. Clamp connections on all the diploid mycelia of these *Collybia* species are large and easily seen. Here also, there is a noticeable difference in the rate of growth of haploid and diploid mycelia. In the culture shown on plate 36, figure 10, the large haploid mat (three weeks old) had a radius of 1.4 cm. when inoculated at the periphery with a small piece of the complementary mycelium. During the five weeks following this inoculation, the original haploid mat grew 2.1 cm. in radius on the side away from the inoculation, showing that the haploid mycelium continued to grow at its customary rate. The diploid mycelium, during the same five weeks, extended radially to a distance of 5.5 cm., gradually encompassing the haploid. At the time the photograph was taken, the haploid mycelium retained its typical haploid appearance, was powdery with abundant oidia, and no clamp connections could be found anywhere upon that area.

Haploid mycelia originating from basidiospores of the same pileus showed great variation in the rate of growth. Some were very slow, attaining a diameter of only 2 cm. in two weeks at 20° C., while others grew to 7 cm. in diameter under the same conditions during the same period.

In all the haploid mycelia of the three species of *Collybia* studied, oidia were produced, generally in large quantities, formed by the breaking-up of the mycelium into individual cells which vary in length. In the slower growing haploid mycelia, practically the whole mycelial mass breaks up into oidia, while in

the faster growing haploids, the oidia make up a much smaller proportion of the mat. In both cases, however, the oidia are responsible for the opaque powdery appearance of the mycelium. The writer has never seen oidia on the diploid mycelia of the *Collybia* species studied.

Diploid mycelia of the three species of *Collybia* are easily distinguishable from each other. When the diploid mycelium of *C. tuberosa* is seven to ten days old, white beaks or small horns push out from the oldest part of the mat. These are accompanied by the watery exudate so characteristic of sclerotia formation (PLATE 36, FIG. 1). These horns are sclerotia, but under the conditions of culture they do not become hard and horny as in nature, and only a few take on the dark reddish brown color (PLATE 36, FIG. 8). The dark colored sclerotia do not produce fruit bodies as quickly as those which remain light colored. In the field, the sclerotia are clearly delimited from the stipe. In culture the sclerotium and stipe are not distinct from each other. Some of the sclerotia in culture show a rudimentary pileus at a very early age, without a well differentiated stipe (PLATE 36, FIG. 8). Later the sclerotium elongates and the pileus expands (PLATE 36, FIG. 7). These sclerotial "horns" or "beaks" are very sensitive to light, bending directly toward it (PLATE 36, FIG. 8). When the sclerotia-like bodies are injured or cut, there is a marked tendency to proliferate.

The diploid mycelium of *C. cirrata* differs from those of the other two species in having no sclerotium. It is a whitish mouldy growth with no special feature of identification (PLATE 36, FIG. 3).

*C. cirrata* var. *Cookei* is identified by its large irregular yellow sclerotia which soon form on the diploid mycelium. A large sclerotium or group of sclerotia develops in the oldest part of the mat first (PLATE 36, FIG. 2), and later as the mycelium extends, a ring of sclerotia may form at some distance out from the center. Large drops of watery exudate collect around the developing sclerotia (PLATE 36, FIG. 6 AND 10).

Sclerotia are also produced from haploid mycelia of *C. cirrata* var. *Cookei*, but in *C. tuberosa* no sclerotia have been seen on the haploid mycelia, although there are frequently drops of water to

be seen in the central part of the haploid mycelia, suggesting an attempt to form sclerotia.

EFFECT OF TEMPERATURE ON GROWTH. A study was made with regard to the effect of temperature on the rate of growth of diploid mycelia of four species of *Marasmius* and three of *Collybia*. These were grown in petri dishes in triplicate, using Kauffman's nutrient agar. The cultures were grown at nine different temperatures. The results are given in the following table:

TABLE I

AVERAGE GROWTH IN MM. OF DIPLOID MYCELVIA OF SPECIES OF *Marasmius* AND *Collybia* AT END OF TENTH DAY AT VARIOUS TEMPERATURES

Species	Temperatures in Degrees Centigrade								
	3	5	10	14	20	25	29	35	40
<i>M. alliatus</i> .....	0	0	13	18	37	38	42	18	0
<i>M. capillaris</i> .....	0	1	13	34	48	52	0	0	0
<i>M. elongatipes</i> .....	2	6	35	43	58	41	7	0	0
<i>M. epiphyllus</i> .....	8	14	36	48	65	46	1	0	0
<i>C. cirrata</i> .....	2	4	0	7	23	19	0	0	0
<i>C. cirrata</i> var. <i>Cookei</i> .....	5	14	17	23	39	21	0	0	0
<i>C. tuberosa</i> .....	4	8	11	16	33	15	0	0	0

From this table it is evident that there is a decided difference in the reaction of the species to temperature. The lower temperatures inhibited the growth of *M. alliatus* and *M. capillaris* much more than *M. elongatipes*, *M. epiphyllus* and the three species of *Collybia*. *M. alliatus* was outstanding for its ability to develop at the higher temperatures. For *M. elongatipes*, *M. epiphyllus*, *C. cirrata*, *C. cirrata* var. *Cookei* and *C. tuberosa*, the optimum temperature lies at about 20° C., whereas for *M. capillaris* it is probably between 20° C. and 25° C. The sudden cessation of growth between 25° C. and 29° C. leads one to believe that perhaps the optimum lies somewhat below 25° C. *M. alliatus* has a rather high optimum temperature, probably somewhere between 25° C. and 29° C., judging from the rapid decrease in the amount of growth at temperatures above 29° C. Compared at their respective optimum temperatures, *M. epiphyllus* was the most rapidly growing species.

The rate of growth of each of the species of *Collybia* appears to be quite distinct, with *C. cirrata* var. *Cookei* growing most

rapidly of the three, while *C. cirrata* grew most slowly. For some reason the three cultures of *C. cirrata* at 10° C. failed to grow, otherwise all three of the species of *Collybia* show a steady increase in growth as the temperature increases to the optimum 20° C.

**SEXUALITY.** The sexual reactions of the fungi were studied in the usual way, *i.e.*, by pairing monosporous mycelia from basidiospores of a single pileus in all possible combinations. The results will be discussed by species.

1. *Marasmius elongatipes* Peck. When monosporous cultures from a single pileus of this species are paired in all possible combinations, three types of reactions are distinguishable. First, a complete mingling of two mycelia (*a*) when of the same sex, originally derived from the same basidiospore, or, in other words, where the genetic constitution is exactly the same in each mycelium; (*b*) when two sexually compatible mycelia are paired.

Second, a tendency for the two mycelia not to mingle but to remain separated from each other by a line of demarcation of greater or less degree. This reaction appeared between any two incompatible mycelia of different sexual groups (PLATE 35, FIG. 2, 3 AND 5), and it was also found to occur between two mycelia of the same sex which originated from different basidiospores (PLATE 35, FIG. 4). Sometimes the line of demarcation appears as a clear zone, and sometimes as a heaping up of mycelium, as in the last mentioned illustration. The writer has not been able to detect any correlation between this "aversion" and any particular sexual factor.

The third type of reaction is a general diploidization of the two mycelia when they are of complementary sexual constitution (PLATE 35, FIG. 6). Here there is a complete mingling, as mentioned above. A diploid mycelium in this species may be brown and felty or white and fluffy. This apparently depends upon the nature of the haploids which unite. Some of the haploid mycelia are brown, and some are pure white. A study of this color factor has shown that wherever two incompatible mycelia are paired, one brown and one white, there is no mingling of the two. Also, wherever a white haploid mycelium is paired with a brown haploid of complementary sex, the resulting diploid mycelium is brown. If two white haploid mycelia of opposite sex are

paired, the resulting diploid mycelium is white. Brown and white mycelia are found in each of the sexual groups. This offers interesting possibilities for a genetical study.

*Marasmius elongatipes* Peck, collection 2. This collection was made in the vicinity of Ann Arbor, Michigan, about Oct. 1, 1931, by E. B. and E. E. Mains (31-897). Monosporous mycelia were obtained (using fresh material) from two different pilei. In the first case 15 spores were isolated, and in the second case 11 were obtained. Within each group pairings were made in every possible combination, with results as given in tables II and III.

TABLE II

*Marasmius elongatipes* PECK. RESULTS OF PAIRING 15 MONOSPOROUS MYCELIA ISOLATED FROM PILEUS 1

	AB	Ab	aB	ab	
	1 6 7 8 14 15	5 17	12	16 18 19	3 4 9
AB	- - - - -	- - - - -	- - - - -	- - - - -	++ +
	- - - - -	- - - - -	- - - - -	- - - - -	++ +
	- - - - -	- - - - -	- - - - -	- - - - -	++ +
	- - - - -	- - - - -	- - - - -	- - - - -	++ +
	- - - - -	- - - - -	- - - - -	- - - - -	++ +
	- - - - -	- - - - -	- - - - -	- - - - -	++ +
Ab	- - - - -	- - - - -	- - + + +	- - - - -	- - - -
	- - - - -	- - - - -	- + + + +	- - - - -	- - - -
aB	- - - - -	- - - - -	- + + - -	- - - - -	- - - -
	- - - - -	- - - - -	- + + - -	- - - - -	- - - -
	- - - - -	- - - - -	- + + - -	- - - - -	- - - -
	- - - - -	- - - - -	- + + - -	- - - - -	- - - -
ab	+ + + + +	+ - - - -	- - - - -	- - - - -	- - - -
	+ + + + +	+ - - - -	- - - - -	- - - - -	- - - -
	+ + + + +	+ - - - -	- - - - -	- - - - -	- - - -

+, clamp connections formed  
-, no clamp connections formed

The monosporous mycelia of pileus 2 were tested against monosporous mycelia 1, 5, 12 and 3 from pileus 1, in order to assign them to their proper sexual group. The species is clearly tetrapolar in the arrangement of its sexual factors. Of the total 26 haploid mycelia, seven had the genetic constitution AB, eight were Ab, five were aB and six were ab.

*Marasmius elongatipes* Peck, collection 32. This group of fruit bodies was collected in Saginaw Forest, Ann Arbor, Michigan, on Nov. 20, 1931, by E. B. and E. E. Mains (31-898).

TABLE III

*Marasmius elongatipes* PECK. RESULTS OF PAIRING 11 MONOSPOROUS MYCELIA ISOLATED FROM PILEUS 2

	AB	Ab	eB	eb								
	33	21	25	27	31	34	35	28	20	23	32	
AB	33	—	—	—	—	—	—	—	+	+	+	
	21	—	—	—	—	—	—	—	+	—	—	
Ab	25	—	—	—	—	—	—	—	+	—	—	
	27	—	—	—	—	—	—	—	+	—	—	
Ab	31	—	—	—	—	—	—	—	+	—	—	
	34	—	—	—	—	—	—	—	+	—	—	
eB	35	—	—	—	—	—	—	—	+	—	—	
	28	—	+	+	+	+	+	+	—	—	—	
ab	20	+	—	—	—	—	—	—	—	—	—	
	23	+	—	—	—	—	—	—	—	—	—	
	32	+	—	—	—	—	—	—	—	—	—	

Fifteen spores were isolated from one of the pilei while fresh, and the monosporous mycelia obtained from them were paired in all possible combinations, with results as given in table IV.

This table also shows the species to be tetrapolar. The twenty-six monosporous mycelia obtained from collection 2 were paired with four tester mycelia from collection 32, namely mycelia 7, 15, 26 and 2. Of the 104 pairings, 103 showed clamp connections. The other culture became contaminated. These results compare well with similar data submitted by Hanna (10) for *Coprinus lagopus* where complete interfertility was the rule in such crossings.

Pennington (19) gives the size of the basidiospores in this species as  $7-8 \times 3.5 \mu$ . Collection 63, made by the writer at Arms Lake, Michigan, Oct. 27, 1932, had spores which measured  $8.9-9.9$  ( $10.5$ )  $\mu \times 3.3-3.6 \mu$  when selected at random. The basidia bore four spores, so the larger spore size cannot be attributed to the number of spores produced as has been found by Smith (21) for two-spored forms of *Mycena*.

TABLE IV

*Marasmius elongatipes* PECK. RESULTS OF PAIRING 15 MONOSPOROUS MYCELIA ISOLATED FROM ONE PILEUS OF COLLECTION 32

	AB					Ab					aB			ab		
	1	3	7	14	19	4	13	15	17	20	18	25	26	2	12	
AB	-	-	-	-	-	-	-	-	-	-	-	-	-	++		
	-	-	-	-	-	-	-	-	-	-	-	-	-	++		
	-	-	-	-	-	-	-	-	-	-	-	-	-	++		
	-	-	-	-	-	-	-	-	-	-	-	-	-	++		
	-	-	-	-	-	-	-	-	-	-	-	-	-	++		
Ab	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	++	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	++	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	++	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	++	-	-
aB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ab	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-

This was such a striking deviation from the published size that it was considered desirable to pair monosporous mycelia of the long-spored form with monosporous mycelia of collections 2 and 32, both of which had spores agreeing very well with the size given by Pennington. Unfortunately, only one of the original monosporous mycelia from collection 63 survived in culture, so that it became necessary to hunt the long-spored form anew in the fall of 1933. As collection after collection was brought in and measured, it appeared that there were all variations in basidiospore size ranging from the size given by Pennington up to that found by the writer in the collection which came from Arms Lake. In order to study this variation, several collections of *M. elongatipes* were made in close proximity to each other at Cascade Glen, near Ann Arbor, Michigan, on Oct. 23, 1933. Mature pilei were selected from these collections and allowed to deposit their spores in sterile capsules. The pilei were selected from groups growing within a few feet or at most a few yards of each other, and were labelled A, B, C, D and E respectively. Twenty spores

were selected at random from a spore print from each of the pilei, with results as given in table V.

TABLE V

*Marasmius elongatipes* PECK. SHOWING VARIATION IN LENGTH OF BASIDIO-SPORES FROM FIVE COLLECTIONS OF THE SAME SPECIES

Pileus	Range of length of spores	Average length
A	7.6- 9.0	8.3
B	7.6- 9.6	8.6
C	5.6- 8.5	7.0
D	7.4-11.2	9.3
E	7.6-10.1	8.8

From these figures it is seen that the two populations C and D are extremes, each occupying a distinct range which only slightly overlap each other. The other populations occupy a range slightly intermediate between those of C and D.

The sexual reaction of the original long-spored form (collection 63) from Arms Lake was determined by mating 8 monosporous mycelia in the usual way. The results of these pairings are given in table VI.

TABLE VI

*Marasmius elongatipes* PECK. RESULTS OF PAIRING 8 MONOSPOROUS MYCELIA OF THE LONG-SPORED FORM, COLLECTION 63

	AB	Ab	-	aB				
	6	9	1	3	13	14	4	7
AB	6	-	-	-	-	-	-	-
	9	-	-	-	-	-	-	-
	1	-	-	-	-	-	++	-
Ab	3	-	-	-	-	-	++	-
	13	-	-	-	-	-	++	-
	14	-	-	-	-	-	++	-
	4	-	-	++	++	++	-	-
aB	7	-	-	++	++	++	-	-

The long-spored form exhibits the tetrapolar arrangement of sexual factors as does the short-spored form, although in the eight spores isolated in this instance, only three of the sexual groups are represented.

Unfortunately only one of these monosporous mycelia survived, but it was paired with eight monosporous mycelia from each of the short-spored collections 2 and 32, and showed complete fertility with all of them. The eight monosporous mycelia from collections 2 and 32 contained two representatives of each of the four sexual groups in each case. See tables VII and VIII.

TABLE VII

*Marasmius elongatipes* PECK. RESULTS OF PAIRING HAPLOID MYCELIUM 4  
(GENETIC CONSTITUTION AB) OF THE LONG-SPORED COLLECTION 63  
WITH 8 HAPLOID MYCELIUM OF SHORT-SPORED COLLECTION 2

	AB	Ab	aB	ab
2	1	6	5	21
63	12	16	3	4
4	+	+	+	+
	+	+	+	+
	+	+	+	+
	+	+	+	+
	+	+	+	+

TABLE VIII

*Marasmius elongatipes* PECK. RESULTS OF PAIRING HAPLOID MYCELIUM 4  
(GENETIC CONSTITUTION AB) OF THE LONG-SPORED COLLECTION 63  
WITH 8 HAPLOID MYCELIUM OF SHORT-SPORED COLLECTION 32

	AB	Ab	aB	ab
32	1	3	4	13
63	18	25	2	12
4	+	+	+	+
	+	+	+	+
	+	+	+	+
	+	+	+	+

Complete fertility was also found when nine monosporous mycelia of known sexual reaction, from the long-spored population D, were paired with four haploid mycelia of collection 2 and four of collection 32. The results of these pairings are given in tables IX and X.

From these data it is seen that the long-spored forms behave like the other collections of this species. The description of the species should be amended to read "basidiospores very variable as to length, 6.6–11, averaging 7–9.7 by 3.3–3.6 in width."

As has been pointed out above, the situation in this species is comparable to that in *Coprinus lagopus*, where Hanna (10) discovered that between different strains of the fungus there is complete fertility.

2. *Collybia cirrata* FRIES. The material for this study was collected by the writer in Saginaw Forest, Ann Arbor, Michigan, Oct. 1, 1932 (3220). In this region it was quite abundant, grow-

TABLE IX

*Marasmius elongatipes* PECK. RESULTS OF PAIRING 9 MONOSPOROUS MYCELIA OF LONG-SPORED POPULATION D WITH 4 MONOSPOROUS MYCELIA OF SHORT-SPORED COLLECTION 2

TABLE X

*Marasmius elongatipes* PECK. RESULTS OF PAIRING 9 MONOSPOROUS MYCELIA OF LONG-SPORED POPULATION D WITH 4 MONOSPOROUS MYCELIA OF SHORT-SPORED COLLECTION 32

TABLE IX

	AB	Ab
D	2	1 6 5 21
AB	1	++ + + +
	5	++ + + +
	6	++ + + +
	7	++ + + +
	9	++ + + +
Ab	3	++ + + +
	8	++ + + +
ab	2	++ + + +
	4	++ + + +

TABLE X

	AB	Ab
D	32	1 3 4 13
AB	1	++ + + +
	5	++ + + +
	6	++ + + +
	7	++ + + +
	9	++ + + +
Ab	3	++ + + +
	8	++ + + +
ab	2	++ + + +
	4	++ + + +

ing amongst pine needles on the withered remains of *Boletus luteus*. This species of *Collybia* does not develop sclerotia. The base of the stipe is provided with very characteristic strigose hairs, and is distinctly rooting.

At first, only eight monosporous mycelia were obtained from this species, and they were paired together to ascertain the sexual conditions. The results of these pairings are given in table XI.

TABLE XI

*Collybia cirrata* FRIES. RESULTS OBTAINED BY PAIRING 8 MONOSPOROUS MYCELIA IN ALL POSSIBLE COMBINATIONS

	AB	Ab	ab
	1 4 7 2 6 8 3 5		
AB	1	-- -- -- -- -- ++	
	4	-- -- -- -- -- ++	
	7	-- -- -- -- -- ++	
Ab	2	-- -- -- -- -- --	
	6	-- -- -- -- -- --	
	8	-- -- -- -- -- --	
ab	3	++ + -- -- -- --	
	5	++ + -- -- -- --	

Since the reaction showed a clear case of tetrapolarity in which the sexual group  $aB$  was not represented, 23 additional spores were isolated and tested against three of these mycelia showing different sexual reaction. These results are given in table XII.

TABLE XII

*Collybia cirrata* FRIES. DETERMINATION OF SEX OF 23 ADDITIONAL MONOSPOROUS MYCELIA

	AB				Ab				aB				ab										
	9	13	27	33	42	17	40	11	18	21	23	29	31	20	22	25	26	37	38	39	43	44	45
AB {	1	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+
Ab {	2	—	—	—	—	—	—	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—
ab {	3	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Combining the two groups of monosporous mycelia isolated, it was found that of the total 31 mycelia, 8 had the genetic constitution AB, 5 were Ab, 6 were aB and 12 were ab.

3. *Collybia cirrata* Fries var. *Cookei* Bres. This form was collected by the writer upon humus in the woods near Duck Lake, Pinckney, Michigan, Sept. 24, 1932 (321). It has also been collected by A. H. Smith, who found it in the vicinity of Ann Arbor, Michigan, Oct. 6, 1932, growing upon soil, and by E. B. Mains, who found some on soil and some on agaric material at Lakeland, Michigan, Oct. 15, 1932 (32-879). In all cases the form is readily identified by the presence of a yellow nodular sclerotium at the base of the stipe. Seventeen monosporous mycelia were obtained and paired with each other as illustrated in table XIII.

In this case the inhibition factor or factors do not appear to be connected with any of the factors for sex determination, and lines of demarcation were frequently found between mycelia of the same sex but originating from different spores.

The proportions of the different sexual groups being so unequal in this experiment, 13 more spores were isolated and their mycelia tested as to sex. The results are given in table XIV.

Thus, in the 30 monosporous mycelia isolated altogether for this species, 5 had the constitution AB, 10 were Ab, 9 were aB and 6 were ab, giving another example of tetrapolarity.

Three types of reactions were observed here as in the case of *Marasmius elongatipes*, when haploid mycelia were paired. The first type of reaction, a mingling of the two mycelia, is illustrated on plate 36, figure 5. The second type, where a line of demarcation is formed between two mycelia, is illustrated on plate 36.

TABLE XIII

*Collybia cirrata* FRIES VAR. *Cookei* BRES. RESULTS OF PAIRING 17 MONOSPOROUS MYCELIUM IN ALL POSSIBLE COMBINATIONS

**c. contamination prevented observation**

∴ inhibition, i.e., distinct line between mycelia.

±, clamp connections present

= clamp connections not formed

TABLE XIV

*Collybia cirrata* FRIES VAR. *Cookei* BRES. DETERMINATION OF THE SEX OF  
13 ADDITIONAL MONOSPOROUS MYCELIUM

figure 6, while the third type, the diploidization reaction, is illustrated in figure 4 of the same plate, and presents a very different picture to that found in *Marasmius elongatipes*.

4. *Collybia tuberosa* Fries. Specimens of this species were collected in the summer of 1932 on decayed agaric material in a swamp at Rock River, Michigan, by E. B. and E. E. Mains (32-184). That fall, it was also collected by the writer at Oscoda, Michigan (3266), where it was growing upon decayed agaric material and also amongst pine needles where decayed agaric

TABLE XV

*Collybia tuberosa* FRIES. RESULTS OF PAIRING 17 MONOSPOROUS MYCELIA IN ALL POSSIBLE COMBINATIONS

	AB				Ab				ab								
	1	4	9	13	15	22	5	18	27	10	16	21	23	25	28	29	31
AB	-	-	-	-	-	-	-	-	-	+	+	+	c	+	-	+	c
	-	c	-	*	-	-	-	-	-	+	c	+	+	+	+	+	+
	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+
	-	*	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
Ab	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
- ab	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ab	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

\* Clamp connections began to form but did not completely fuse. Appeared as small curved branches only occasionally bridging over septum to touch neighboring cell, presumably "Pseudoschnallen" as described by Bruns-wik (5).

† These exceptions were very carefully checked, but no clamp connections were found. The apparent sterility in these cases can not be due to physiological immaturity of the mycelia, as they form clamp connections with other complementary mycelia. It may be due to an insufficient difference in the sexual "realisateurs" of Wettstein as Vandendries (23) suggests in similar instances.

material was not evident. The most characteristic feature of this species is its small dark reddish brown sclerotium, which is shaped somewhat like an elongated and rather shrivelled apple seed. It appeared to be growing upon withered pilei of *Russula*, but it was not possible to determine whether it is limited to this genus.

The results of pairing 17 monosporous mycelia of this species are given in table XV.

ATTEMPTS TO PRODUCE INTERSPECIFIC CROSSES. Since the three forms of *Collybia* used in this study appear to be closely related to each other, it seemed desirable to use the material in an attempt to obtain crosses between them. Tables XVI, XVII and XVIII embody the results of such experiments.

TABLE XVI  
THE RESULTS OF PAIRING REPRESENTATIVES OF THE FOUR SEXUAL GROUPS  
OF *C. tuberosa* WITH THOSE OF *C. cirrata*

		<i>C. tuberosa</i>					
		AB	Ab	ab	ab	10	25
C. cirrata	AB	13	15	5	18	27	10
	AB	33	—	—	—	—	—
	AB	42	—	—	—	—	—
	AB	2	—	—	—	—	—
	AB	6	—	—	—	—	—
	AB	21	—	—	—	—	—
	AB	23	—	—	—	—	—
	ab	39	—	—	—	—	—
	ab	44	—	—	—	—	c
	ab	—	—	—	—	—	—

—, no clamp connections formed; c, culture contaminated

TABLE XVII  
THE RESULTS OF PAIRING REPRESENTATIVES OF THE FOUR SEXUAL GROUPS  
OF *C. tuberosa* WITH THOSE OF *C. cirrata* VAR. *Cookei*

		<i>C. tuberosa</i>					
		AB	Ab	ab	ab	10	25
C. cirrata	var. Cookei	13	15	5	18	27	10
	var. Cookei	37	—	—	—	—	—
	var. Cookei	40	—	—	—	—	—
	var. Cookei	19	c	—	—	c	—
	var. Cookei	22	—	—	—	—	—
	var. Cookei	12	—	—	—	—	—
	var. Cookei	30	—	—	—	—	—
	var. Cookei	39	—	—	—	—	—
	var. Cookei	43	—	—	—	—	—
	var. Cookei	—	—	—	—	—	—

—, no clamp connections formed; c, culture contaminated

TABLE XVIII

THE RESULTS OF PAIRING REPRESENTATIVES OF THE FOUR SEXUAL GROUPS.  
OF *C. cirrata* WITH THOSE OF *C. cirrata* VAR. *Cookei*

		<i>C. cirrata</i> sp.							
		AB	Ab	ab	ab				
		1	4	2	6	9	18	3	5
<i>C. cirrata</i> var. <i>Cookei</i>		2	—	—	—	—	—	—	—
		10	—	—	—	—	—	—	—
		19	—	—	—	—	—	—	—
		17	—	—	—	—	—	—	—
		8	—	—	—	c	—	—	—
		9	—	—	—	—	—	—	—
		45	—	—	—	—	—	—	—
		44	—	—	—	—	—	—	—

The results of the foregoing attempts to produce interspecific crosses have all been negative. Complete sterility appears to exist between these forms. Kniep (14) reported an experiment which he performed in connection with his studies upon the sex of *Collybia cirrata* Fries which is interesting in this regard. He obtained from two localities in Brandenburg (Havelberg and Finkenkrug) what he thought to be two collections of *C. cirrata*. He paired them, and found them to be completely intersterile. His statement (14, p. 414) reads as follows: "Die kleinen charakteristischen, in grossen Mengen auf verrotteten Pilzen auftretenden Fruchtkörper beiderlei Herkunft stimmt völlig miteinander überein, ebenso die Sporen und die daraus kultivierten Myzelien, die durch einen äusserst auffallenden Erdgeruch ausgezeichnet sind. Der einzige Unterschied war, dass der Pilz von Finkenkrug sehr stark zur Sklerotienbildung neigte, der andere nicht. Die Haplonten beider Pilze waren gegeneinander völlig steril."

It is evident that he was dealing with *Collybia cirrata* Fries and *C. cirrata* Fries var. *Cookei* Bres.

METHODS OF DIPLOIDIZATION. The species of fungi studied in this investigation present two distinct types of diploidization. One type is found in *Marasmius elongatipes*, the other type is exhibited by the three species of *Collybia*. In *Marasmius elongatipes* diploidization occurs in the same manner as that described by Buller (6) for *Coprinus lagopus*. In this type, when two haploid mycelia of complementary sexual constitution

are paired, the diploid condition arises first at the line of contact of the two mycelia and spreads from there through both of the original haploid mycelia, progressing more rapidly in the younger parts of these mats. It is very difficult to see any macroscopic differences between haploid and diploid mycelia of *M. elongatipes*, if one leaves out of consideration the difference in the rate of growth of haploid and diploid mycelia. The method recommended by Buller (6) for studying diploidization, namely that of inoculating a large mat of a haploid mycelium with a small piece of a complementary mycelium at the periphery, brought out clearly the difference in the two kinds of diploidization. In plate 36, figure 6, a large mat of haploid mycelium 7 of *Marasmius elongatipes*, with the genetic constitution AB, was inoculated at a point on its margin with a small piece of haploid mycelium 2, whose genetic constitution was ab. At the time the photograph was taken, two weeks later, the culture was examined and it was seen that the diploid condition had spread through much of the haploid mycelium 7. The extent of the diploid condition, as determined by the presence of clamp connections, is shown by the letter d, while the areas in which clamp connections were not found are marked h, presuming that such areas are still haploid.

The three species of *Collybia* showed a very different phenomenon from that of *Marasmius elongatipes*. In this case, when two complementary haploid mycelia were paired, the diploid mycelium formed along the line of contact, but the mycelia originally haploid remained so. The diploid mycelium grows so much faster than the haploid that it soon grows out and surrounds the latter. It is easy to tell even macroscopically which is haploid and which is diploid mycelium (PLATE 36, FIG. 4). Large haploid mycelia were inoculated with a small amount of their complementary mycelium placed at a point on their margin, and the manner of diploidization is shown on plate 36, figure 10. The haploid mycelium remains haploid and retains the opaque powdery appearance characteristic of the haploid mycelia of this group, while the diploid mycelium forms as a fan, gradually surrounding the haploid, and easily distinguishable from it by the possession of rather coarse radiating hyphae which are quite conspicuous. This might be regarded as a sort of "limited diploidization," for

there is certainly no progressive transformation of haploid into diploid mycelium in this case. This "limited diploidization" may be similar to that which Vandendries (26) has recently reported in *Trametes suaveolens*.

STUDIES CONCERNING MYCELIAL FUSIONS. Davidson, Dowding and Buller (7) made careful observations on the formation of fusions between different mycelia in their study upon dermatophytes. They found that fusions would occur only between mycelia of the same species, and were enabled thereby to identify species of dermatophytes much more quickly than before. Vandendries (28) has found that this is true also for basidiomycetes, and that all mycelia of one and the same species are capable of forming mycelial fusions with each other, even though they may be sexually incompatible haploids. It seemed advisable to subject the three forms of *Collybia* concerned in this study to such a test. Diploid mycelia of *C. cirrata* were paired with diploid mycelia of *C. cirrata* var. *Cookei* on an agar film in Van Tieghem cells as described by Davidson, Dowding and Buller (7). Similar pairings were made of diploid mycelia of *C. cirrata* and *C. tuberosa*, and of *C. cirrata* var. *Cookei* and *C. tuberosa*. Pairings were also made between two diploid mycelia of the same species in each case, e.g., *C. tuberosa* with *C. tuberosa*. There were numerous fusions observed between mycelia of the same species, and between different branches of the same mycelium, but no mycelial fusions were noted in which two mycelia of different species were involved. Neither were there any fusions between the diploid mycelia of *C. cirrata* and *C. cirrata* var. *Cookei*. In this instance, moreover, it was observed that *C. cirrata* var. *Cookei* seemed to be adversely affected when its hyphal tips penetrated into the edge of the area covered by the mycelium of *C. cirrata*, for its tip branches became distorted and gnarled, and produced several unnaturally short and stunted branchlets at the end of the hyphae. No such reaction occurred between *C. cirrata* and *C. tuberosa*, or between *C. cirrata* var. *Cookei* and *C. tuberosa*.

SPOROPHORE PRODUCTION UPON ARTIFICIAL MEDIA. *Marasmius elongatipes* Peck. (PLATE 35, FIG. 1) fruited readily upon several media including malt agar, medium 5, medium 10, Kauffman's nutrient agar, and modifications of Poole's medium

and Etter's medium. On Kauffman's agar the fruit bodies were not normal, and did not produce spores. On all the other media the sporophores were practically normal, except that the stipe in some cases was thicker than it is in nature. From three of the compatible matings between collections 2 and 32 of this species eight small sporophores were produced in the petri dishes.

*Marasmius capillaris* Morg. fruited upon medium 5, while *M. epiphyllus* Fries fruited upon medium 5 and medium 10.

*Collybia cirrata*, *C. cirrata* var. *Cookei* and *C. tuberosa* all fruited on various media which contained as a base the dried pilei of *Boletus luteus*. Although *C. tuberosa* is reported as usually growing upon remains of species of *Russula*, and *C. cirrata* var. *Cookei* generally on soil, it was interesting to see them thrive on *Boletus luteus*, the substratum on which *C. cirrata* grows in this region.

An attempt was made to grow the species of *Marasmius* upon the *Boletus* medium, but not the slightest trace of growth of these species could be obtained on this material.

None of the above-mentioned species produced fruit bodies when grown in the dark, nor have sporophores been produced by any of the haploid cultures up to the present time.

LONGEVITY AND ABILITY OF PILEI TO REVIVE. From time to time during the course of this study, attempts were made to obtain spore deposits from revived pilei of various species of *Marasmius* and *Collybia*. *Marasmius elongatipes*, *M. delectans*, *M. alliatus*, *M. urens* and *M. rotula* all gave negative results. They regained their normal shape and texture, but deposited no spores. *Collybia cirrata* var. *Cookei* and *C. tuberosa*, however, gave better results. *C. cirrata* var. *Cookei* when revived after 51 days of storage in a dry condition gave a good spore deposit when suspended over an agar plate, and *C. tuberosa*, after 86 days in a dried state, also yielded a good spore print. Moreover, the spores in both instances were viable. In each case, two out of four revived pilei deposited their spores readily.

DISCUSSION. Two different types of diploidization have been noted in these studies. Buller (5), in his work upon *Coprinus lagopus*, found that when two monosporous mycelia of complementary sexual constitution were paired, the diploid condition

formed first at the line of contact of the two mycelia and subsequently spread throughout both mycelia, advancing more rapidly through the young hyphae than through the older. This he believed due to the passage of rapidly-generated daughter nuclei of the one mycelium travelling through the other mycelium and becoming associated with the nuclei of the mycelium through which they were passing to form pairs of nuclei which thereafter would divide conjugately. As has been shown, *Marasmius elongatipes* behaves very much the same as *Coprinus lagopus*.

In the three species of *Collybia* studied, an entirely different kind of diploidization prevails. Here there is no spread of the diploid condition through or across the paired haploids. The diploid cells arise merely at the line of contact, and from them diploid hyphae develop and spread out in fans from between the two haploid mycelia. On account of its greater rapidity of growth, the diploid mycelium grows around the two haploids, but in doing so, it encroaches little, if any, upon them. In the culture shown on plate 36, figure 10, it can be seen that the haploid mycelium continued to grow at its usual rate even after the diploid mycelium had begun to develop.

While *Collybia cirrata*, *C. cirrata* var. *Cookei* and *C. tuberosa* all present this "limited diploidization," it is interesting to note that H. J. Brodie (4) has reported that *C. velutipes* Fries has a type of diploidization resembling that of *Coprinus lagopus*.

Several cases have been reported where the diploid mycelium appears to be limited to a narrow region along the line of contact. Vandendries (26) has made mention of this condition in *Trametes suaveolens*. The same author in collaboration with H. J. Brodie has recorded it also in *Polystictus versicolor*, and Smith and Brodie (22) in an article on *Pholiota polychroa* (Berk.) Smith & Brodie have pointed out the same fact. In all of these cases, however, there appears to be considerable growth of the haploids before there is any sign of diploid mycelium forming, and when it does appear, the diploid mycelium does not seem to have the greatly accelerated rate of growth that it has in the case of the *Collybia* species. In all of these cases also the authors report that the diploid mycelium grows across or over the haploid, the diploid and haploid mycelia mingling until finally there is great

difficulty in finding a trace of the original haploid mycelia. This is quite different from the situation in the three species of *Collybia* described in the present paper, although in all these cases the diploidization might be described as strictly "limited."

The variations in reactions between different haploid mycelia of a single species when paired together present perplexing questions. "Lines of demarcation," "inhibition," "Hemmungsreaktion," "aversion," "repulsion" and "barrage" are terms which have been used by different investigators to denote various types of incompatibility.

Some investigators have found in the results of their experiments very clear cut reactions. Vandendries (25) found that "barrage" was dependent on certain combinations of Mendelian factors, and traced a distinct correlation between the occurrence of "barrage" and the common possession of the a or a' factor by the two paired mycelia. He maintains (27) that this "barrage sexuel" cannot exist in bipolar species, and must not be confused with the phenomena of demarcation which do not behave in a Mendelian fashion.

In *Marasmius elongatipes* it is evident that there is something in the nature of an inhibition or demarcation factor which is not associated with the sexual factors. In fact, demarcation between mycelia in this species is more prevalent than mingling of mycelia, for complete mingling only occurred when diploidization took place or when the paired mycelia had originated from the same spore. Even mycelia of the same sexual constitution refused to mingle if they had arisen from different spores. Miss Mounce (17) found in her studies on *Fomes pinicola* that lines of demarcation were very general between monosporous (and even between diploid) mycelia, and that mingling occurred only between "closely related" mycelia. Oort (18) reported similar conditions in *Coprinus fimetarius* when he distinguished between "OO-Kombinationen" (mycelia from the same basidiospore) and "O-Kombinationen" (mycelia of the same sex but from different basidiospores), and reported that in the latter combinations he sometimes found a weak repulsion.

*Marasmius elongatipes* presents an unusual condition in regard to the variation in size and shape of basidiospores. The spore

size is commonly given as  $7-8 \times 3.5 \mu$  (19) for this species. However, in normally deposited spore prints some spores have been found only  $5.6 \mu$  in length, while others measured as much as  $11.2 \mu$  in length. The width varied very slightly, ranging from  $3.3 \mu$  to  $3.6 \mu$ ; consequently the variation in length involved a great diversity of spore forms as well as spore dimensions. The range of size in the short-spored group slightly overlapped the range of the large-spored group, while other populations were intermediate. When mated, the sexual strains of the long-spored group were completely interfertile with those of the short-spored group, showing that they are races of the same species.

In studying the relationship of *Collybia cirrata* Fries var. *Cookei* Bres. to *C. cirrata* Fries and *C. tuberosa* Fries, all attempts to produce interspecific hybrids yielded only negative results. Microscopic examination showed that there were no mycelial fusions between these species when grown together. The diploid mycelium of *C. cirrata* var. *Cookei* became greatly branched and stunted at the tips of the hyphae when grown along with *C. cirrata*. This was the only case observed where one mycelium had a visible effect on the other. The complete absence of mycelial fusions between the three forms when paired with each other, and the fact that the production of sclerotia was a consistent feature of the diploid mycelia of *C. tuberosa* and *C. cirrata* var. *Cookei*, while sclerotia were never developed in *C. cirrata*, furnish evidence that the agaric hitherto recognized as *Collybia cirrata* Fries var. *Cookei* Bres.<sup>2</sup> is in reality a specific entity.

All three species of *Collybia* studied showed the same type of oidia on the haploid mycelia. No oidia were observed on diploid mycelia of these species of *Collybia*. On *Marasmius elongatipes* no oidia were produced either on the haploid or on the diploid mycelia.

The power of revival has long been considered a diagnostic character of the genus *Marasmius*. When a dried and shrivelled specimen of *Marasmius* is placed for a time in a moist chamber, there will be a marked return to the normal shape and texture. This was found true not only for the species of *Marasmius* studied, but also for the three species of *Collybia*. Spore deposits

<sup>2</sup> *Collybia Cookei* (Bres.) comb. nov. is the name proposed for the agaric hitherto recognized as *Collybia cirrata* Fries var. *Cookei* Bres.

were not obtained from any of the revived plants of *Marasmius*. On the other hand, revived plants of two of the species of *Collybia* deposited abundant viable spores. Whether the spores of *C. Cookei* and *C. tuberosa* were produced after revival, or whether it was merely a mechanical shedding of spores already formed, was not determined. This evidence would tend to put this group of *Collybia* species into the section *Marasmioideae* instead of the section *Vestipedes* where they have been placed for so many years.

#### SUMMARY

1. During the course of this investigation, the vegetative growth and sexual reactions have been studied for the following fungi: *Marasmius elongatipes* Peck, *Collybia cirrata* Fries, *C. cirrata* Fries var. *Cookei* Bres. and *C. tuberosa* Fries.
2. These were all found to be tetrapolar species.
3. Strains of *Marasmius elongatipes* from different localities in the vicinity of Ann Arbor, Michigan, were completely interfertile with each other when paired.
4. The kind of diploidization presented by *Marasmius elongatipes* was found to agree with the diploidization phenomenon as described by Buller for *Coprinus lagopus*.
5. A different type of sexual reaction was found in the *Collybia* species studied. Here diploidization is localized, being confined to the region of contact of the paired mycelia. Diploid cells form in this region, and from them the diploid mycelium grows, but the original haploid mycelia are not converted into diploid mycelia. For this, the term "limited diploidization" is suggested.
6. Oidia were found to occur on haploid mycelia of the three *Collybia* species studied but not on the haploid mycelia of *Marasmius elongatipes*. No oidia were seen on diploid mycelia of any of the species studied.
7. All attempts to produce hybrids between the three closely related forms of *Collybia* yielded only negative results.
8. The mycelial fusion test for specific identity was used with these species, and no fusions were observed between the three forms.
9. It is therefore concluded that *Collybia cirrata* Fries var. *Cookei* Bres. should be given specific rank, as *Collybia Cookei* (Bres.) J. Arnold.

10. Sporophore production was obtained upon artificial media for *Marasmius elongatipes*, *M. capillaris*, *M. epiphyllus*, *Collybia cirrata*, *C. Cookei* and *C. tuberosa*.

11. Pilei of *Marasmius* species were revived, but no spore deposits were obtained from them.

12. Pilei of *Collybia* species were successfully revived after more than two months in a dried condition. Basidiospores were discharged from them and germinated in the usual time on nutrient agar.

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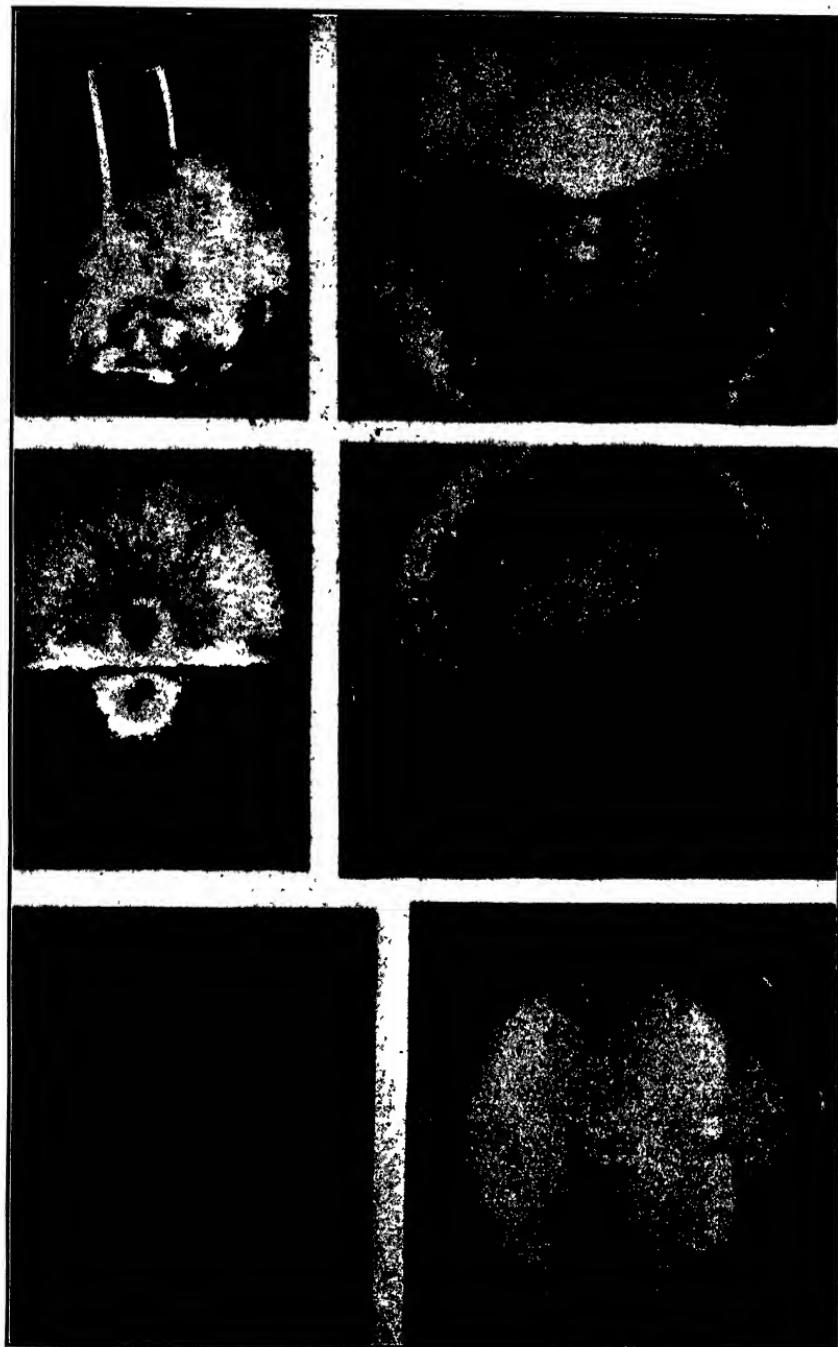
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#### EXPLANATION OF PLATES

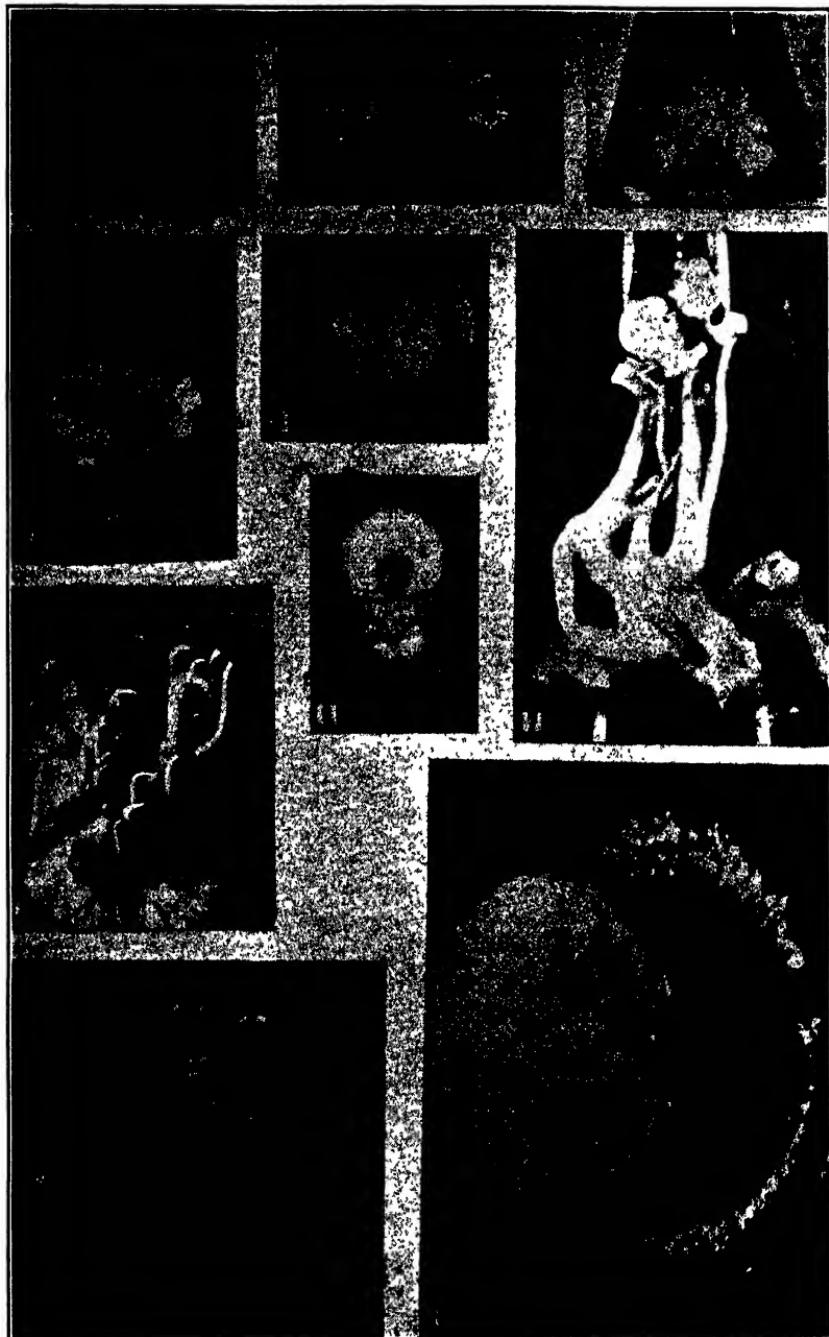
##### PLATE 35

*Marasmius elongatipes* Peck: 1, sporophore development on medium 5; 2, two incompatible haploid mycelia, showing the line of demarcation between them (3 ab X 16 ab); 3, two incompatible haploid mycelia, showing line of



MARASMIUS ELONGATIPES





*COLLYBIA* SP.



demarcation between them (2 ab  $\times$  20 Ab); 4, two mycelia of the same sex but from different basidiospores, showing line separating the two mycelia (8 AB  $\times$  15 AB); 5, two incompatible haploid mycelia, showing line of demarcation between them (14 AB  $\times$  26 aB); 6, diploidization as it occurs when a large haploid mycelium (7 AB) is inoculated at the margin with a complementary haploid mycelium (2 ab). Areas already diploid marked d, areas still haploid marked h.

#### PLATE 36

Fig. 1, *Collybia tuberosa* Fries, showing the development of horn-like sclerotia on *Boletus* extract agar; 2, *Collybia Cookei* (Bres.) Arnold (*C. cirrata* Fries var. *Cookei* Bres.) showing the development of large irregular sclerotia on Kauffman's agar in which *Boletus* extract was used instead of water in the formula; 3, *Collybia cirrata* Fries, development on Kauffman's agar in which *Boletus* extract was used instead of water; 4-6, *Collybia Cookei* (Bres.) Arnold (*C. cirrata* Fries var. *Cookei* Bres.); 4, two haploid mycelia of complementary sexual constitution paired to show how the diploid mycelium forms "Limited diploidization" (15 aB  $\times$  17 Ab); 5, two mycelia from the same spore paired to show complete intermingling (8 aB  $\times$  8 aB); 6, two mycelia of the same sexual constitution but from two different spores paired to show the line of demarcation between them (15 aB  $\times$  16 ab); 7 and 8, *Collybia tuberosa* Fries; 7, development of abnormally large sporophores on *Boletus* pilei laid on top of moist soil; 8, showing sclerotia elongating into stipe, and pilei beginning to differentiate, also showing how sclerotia turn towards light; 9 and 10, *Collybia Cookei* (Bres.) Arnold (*C. cirrata* Fries var. *Cookei* Bres.); 9, development of sporophores on pilei of *Boletus luteus*; 10, diploidization as it occurs when a large haploid mycelium (2 AB) is inoculated at the margin with a complementary haploid (3 ab), diploid mycelium gradually growing around haploid. (Contrast with figure 6 on previous plate.)

# THE MECHANICS OF SEXUAL REPRODUCTION IN NEUROSPORA

B. O. DODGE

(WITH PLATES 37-40)

The course by which a perithecium of *Neurospora* reaches full maturity is marked by three rather distinct stages of development. First there appears the perithecial fundament, the ascogonium. This is quickly surrounded by several layers of compact hyphal growth to form the incipient ascocarp. Nuclei of opposite sex reaction then come together in the ascogenous cells if these cells do not already contain both kinds, as is normally the case in *N. tetrasperma*. This stimulates further growth of the fruit body, the differentiation of wall tissue, the formation of the ostiolar papilla, and finally the development of ascogenous hyphae. Fertilization is consummated with nuclear fusion in the ascus. This is followed by the reduction divisions and the delimitation of ascospores.

## THE INCIPIENT PERITHECIUM

The ascogonium is of the type usually found in species of the Sordariaceae figured many times in the early literature on the subject. It consists of a rather blunt-ended coiled structure of five or six cells or more. Each cell contains several nuclei. The ascogonium of normal *N. tetrasperma* where the cells are provided with nuclei of both sexes from the start, never differentiates a trichogyne (4). Basal cells and branches from the parent hypha grow out to encompass the ascogonium, surrounding it with a compact tissue of several cell layers (PLATE 37, I).

The ascogonia of the heterothallic species and of the unisexual races of *N. tetrasperma* are all very much like those formed on mycelia of normal bisexual *N. tetrasperma*. They consist of the same blunt-ended coils of two or three irregular turns composed of from five to ten cells and without any trichogynes whatever. Just what the large Y-shaped branches one sees in many sections

(PLATE 38, E) represent is a question. The cells contain several nuclei often arranged in pairs. The pairs represent merely sister nuclei. Sterile haploid hyphal branches interlace to form a compact web about the coil. After the incipient perithecium has reached a certain size just visible to the unaided eye, it usually ceases to increase further if the race is grown by itself. Soon, however, trichogynous elements grow out from certain of the ascogonial cells. These narrow, deeply staining branches burrow out through the surrounding compact sterile tissue, zigzagging as they pass between the cells as though they were the hyphae of some intercellular parasite. They are somewhat like the trichogynes of *Coccomyces* so well described and figured by Backus (3). They are sometimes forced to grow around some distance in a circle before finding a place to emerge (PLATE 37, E). More frequently these hyphae grow out as branches from the end cells of the ascogonium, but they may develop from a more central cell. The tip end may form two or three such receptive branches.

The basal part of the ascogonium is apt very early to send out one or two branches that maintain direct connection with the outside. Such branches take the stain deeply and no doubt can act as receptive elements. They are sometimes partly inclosed by the incipient perithecial tissue (PLATE 38, G). At other times they are free from the point of their origin outward.

Photographs of sections of several incipient perithecia of *Neurospora sitophila* showing the least complicated types of ascogonia are reproduced in plates 37 and 38. These were developed on unisexual mycelia grown separately. The details are given in the plate legends. There can be no doubt that when one applies either the orange-colored monilioid conidia or the microconidia artificially, these trichogynous outgrowths are the means by which nuclei of the other sex are brought into the ascogenous cells. Looking down on plate cultures one often sees these long trichogynes extending out into the air or bending down, their tips fusing with the ordinary vegetative hyphae. No doubt in mixed cultures this would result in fertilization.

At the Sixth International Genetics Congress held in Ithaca, N. Y., 1932, the writer demonstrated with enlarged photographs the course of development of incipient perithecia at six-hour

intervals, beginning with the hour at which the monilioid conidia had been applied to the surface of the young fruit bodies. Two of this series of photographs were published in *Torreya* (11) where it was pointed out that the appearance of the hair-like outgrowths is such as to suggest that they may be receptive hyphae. The photographs exhibited at Ithaca (together with this statement) seem to have misled certain observers into thinking that all such outgrowths must be receptive hyphae. Sections show conclusively that in *Neurospora* most of these delicate hairs grow out from the outermost sterile wall cells and have no direct connection whatever with the ascogonium (PLATE 37, I). It is probably the same in *Pleurorage anserina*.

The details concerning the formation of spermatia or microconidia by species of *Neurospora*, their germination to form perfectly normal mycelia and also their function as fertilizing elements will be found in earlier papers (6-8). It has also been shown that the monilioid conidia serve much more efficiently as spermatizers than do the microspores themselves, and recent experiments indicate that young aerial hyphae, and even trichogynes or trichogynous hyphae if they come in contact with trichogynous elements of the opposite sex reaction, are capable of effecting fertilization. It is not very difficult to make these incipient perithecia fertilize each other.

#### UNISEXUAL MYCELIA UNITED VEGETATIVELY

It remains to determine whether, in mixed cultures of heterothallic races, perithecia mature only when nuclei from outside fertilizing elements such as microspores and conidia enter the ascogonia by way of trichogynes; or may it be that, following vegetative hyphal fusions, there then arise perithecia whose ascogonia are provided with nuclei of both sexes because of nuclear migrations, so that trichogynes are not needed and are not formed.

Köhler (14), who worked rather extensively on anastomoses, found no evidence that in *Neurospora* one could obtain a bisexual mycelium by mixing unisexual conidia of both sexes. The germ tubes would fuse and the regeneration hypha would show a plasma-streaming, but he could not prove a migration of nuclei.

Lindgren (15) without referring to Köhler's work claims on the contrary that such a heterokaryotic condition in *N. crassa* must sometimes result from sowing many ascospores together, but he adds that the bisexual condition may split up again to develop separate unisexual growths. Although one experiment with non-conidial races of *N. sitophila* will be described later on, further work is still necessary before the question as related to normally heterothallic species is settled. The present study is concerned with the same question but more as related to bisexual ( facultatively heterothallic) species such as *N. tetrasperma*, *Pleurage annserina* and *Gelasinospora tetrasperma*. Dowding (12, 13) has shown by her excellent work with the last two species that they are like *Neurospora tetrasperma* in that one can readily obtain unisexual races by germinating the small ascospores. No one has hitherto proved that such unisexual races can, by uniting vegetatively, give rise to the normal bisexual type of mycelium containing both kinds of nuclei.

When our tester races S6 and S1, or S9 and S1, which are unisexual components of *N. tetrasperma*, are planted on opposite sides of a plate culture, the fruit bodies are usually first formed where the mycelia meet, after which they appear one by one along the lines of radiately growing hyphae of the strain S6 (6). The distribution pattern of course varies greatly, depending on culture conditions. In general, however, the perithecia are formed on the S6 side (6, fig. 3, and 7, 1931, pl. 17, fig. 15, 17) and not more on the S1 side as stated by Colson (4). Dowding (13, pl. 11, fig. 11) shows exactly the same distribution of ascocarps in plate cultures of *Gelasinospora*. This suggests at first a differentiation of the sexes, but it probably means merely the manifestation of genetic factors, some of which may possibly be sex-linked. This point will be discussed later on. In any event, why is it that fruit bodies of these species tend to be distributed in this curious fashion, over one side of the plate, often confined to a V-shaped sector at first? There are hundreds of anastomoses along the line of meeting and it looks as though the nuclei coming over from the S1 mycelium may migrate down through the S9 hyphae. In that case the ascogonia of the new incipient perithecia forming on these hyphae, now bisexual because of the

migration, would contain both kinds of nuclei so that the perithecia would go right on and mature. In order to obtain some definite information on this point a number of culture experiments were carried out. A brief outline of the principles involved will facilitate a better understanding of the results to be reported.

The fact that in plating out microconidia and monilioid conidia from the S9 side of plate cultures after perithecia are formed there, one finds all of his isolates belonging to sex B and not any to sex A, or that all are sex A and none sex B, or again that both sexes are represented separately, does not prove anything particularly as regards nuclear migration or intermingling of mycelia. With only nuclear migration operating to bring the other kind of nuclei also into some cells of the S9 mycelium we could still have the split-up again. It is only when we obtain *bisexual* conidia or *bisexual* hyphal tips, do we know that the anastomoses have resulted in bringing together both kinds of nuclei in hyphal cells. In any mixed culture, as previously noted (5, 10), if new branches arise from vegetative fusions to form bisexual conidia, such conidia must be greatly outnumbered by the unisexual conidia already in the culture so that it would be difficult to find them.

#### *Nuclear migration in plate culture*

Tester races S3 and S9 were grown from opposite sides of a plate. Perithecia began to form as usual about the fourth day on the S9 side of the line of meeting. Four small blocks of agar bearing a few very young perithecia were then transferred to separate plates which were incubated for twelve hours. The hyphae grew out so that single hyphal tips could then be isolated very easily. Seven 1-tip tube cultures were obtained from each of the four transplants. Later they all produced perithecia, proving that the single tips isolated must have carried both kinds of nuclei. Since mycelia S3 and S9 with which the plate was inoculated are both unisexual, the bisexual tips isolated later from the S9 side must have represented new branches that grew out from the old S9 mycelium after it had received the S3 nuclei passed along down following anastomoses at the line of meeting. It may be claimed, however, that the bisexual fusion cells result-

ing from anastomoses could proliferate to form a *new* mycelium which would spread out over or through the old unisexual mycelia already in the plate. In such a case there is no reason why this new bisexual mycelium should grow out only over the S9 side. In any case one should obtain at least as many unisexual 1-tip isolates like S9 as he does bisexual tips. It is a very remarkable fact that not one of the twenty-eight 1-tip isolates was unisexual. Furthermore if new bisexual growth does proceed from points of anastomoses one should be able to isolate bisexual conidia very readily from such plates, which is not the case. It is more reasonable to assume that nuclei from the S3 side, following anastomoses with S9 branches, migrate down the S9 hyphae. Ascogonia containing both kinds of nuclei would arise and perithecia would quickly begin to mature.

#### *U-tube cultures*

Conclusive evidence of nuclear migration was also obtained from U-tube cultures being carried on for another purpose. The U-tubes employed in this work were the same that were used by the writer (7), and by Aronescu (1, 2). The procedure is as follows. Two unisexual races such as S1 and S9 are grown from opposite sides of a plate culture. The two mycelia meet and their hyphal tips anastomose. In two or three days one sees the beginnings of perithecia that are destined to mature ascospores because in some way both kinds of nuclei have been brought together in the ascogonial cells. These young perithecia present a different appearance from that of the unfertilized incipient perithecia always formed on unisexual mycelia. A small bit of agar bearing a few young perithecia is transferred to one arm of the U-tube. Within three or four days many more fruit bodies begin to develop in this arm. In the meantime the mycelium will usually have grown down around into the other arm of the U-tube.

The reader is referred to a preceding paper (10) for the pedigree of races used in the following experiments. Unisexual races 38 (9.7C8  $\times$  S9) and 26 (9.7C8  $\times$  S9) both carry the lethal, l, so that perithecia arising from their mating produce asci but no ascospores. Such races are desirable in this work because no

matter how old a culture may be no ascospores can be formed to germinate *in situ* to mislead one. That is, if one grows these two unisexual races in a mixed culture and at any time succeeds in isolating a *bisexual* conidium or a *bisexual* hyphal tip from that mixed culture he can be sure that nuclei originally carried in separate mycelia had come into the same cells following vegetative fusions or anastomoses. This is the particular point proved by the following culture experiments.

Races 38 and 26 (the parental formula 9.7C8 × S9 may be omitted) were grown from opposite sides of a plate culture. Four days later the mycelia had met and perithecia were appearing on the 38 side of the line. A small block of agar carrying a few of the little fruit bodies was transferred to one arm of a U-tube. In due time more perithecia appeared in this arm and the mycelium passed around into the second arm where more conidia and perithecia with asci eventually matured. In no case were any ascospores delimited, because as stated above both parents carry the lethal. About two weeks from the time the culture was started conidia from the second arm were sowed and allowed to germinate slightly. Twelve 1-conidium tube culture isolates were made, choosing the conidia that germinated first. Twenty more 1-conidium isolates were planted in plates to check against the possible accidental transfer of microconidia. The next day twenty-five 1-tip isolates were made from these plate transplants. As a double check the first transplants were again transferred to a harder agar where no water of condensation could possibly carry microspores over on the hyphal tips to be isolated. By the following day the new growth had formed a circle about 2 cm. in diameter in each case. From each of ten such colonies four 1-tip isolates were made with great care. The results obtained in this experiment show that of the twelve 1-conidium cultures first isolated, eight were probably unisexual and sterile and the other four were certainly bisexual and fertile, showing good perithecia. Of the first set of thirty-eight 1-tip cultures, thirty were unisexual and the other eight showed perithecia. The forty-three doubly checked 1-conidium cultures showed about the same percentage of fertility, thirty-five remaining sterile and so must have been unisexual, and eight formed

perithecia and must have been bisexual. Of the ninety-three cultures making up the three sets, seventy-three remained sterile, and twenty were fertile; therefore their mycelia must have been bisexual. This proves conclusively again that a bisexual mycelium can be obtained as the result of a vegetative union or anastomosing of hyphae from two unisexual mycelia of opposite sex reaction.

Twenty-two of the sterile isolates mentioned above were tested for their sex reaction. All of them were sex B, the same as the no. 26 parent which carries the factor O for orange-colored masses of conidia. The no. 38 parent produces only a few conidia comparatively, so that out of the small number one might not obtain any that would be of reaction type A. As a further check, conidia produced in one of the 1-conidium fertile cultures described above were then sowed, and fifty-one 1-conidium isolates were obtained by Dr. S. M. Pady, to whom the writer is indebted for assistance in checking this work. Twenty-five proved to be bisexual, and twenty-six unisexual and sex B in their reaction. No doubt one could build up a still higher percentage of bisexuality by selection.

The same type of U-tube experiment was then performed but using the unisexual parents 43 (9.7C8  $\times$  S9) and T100 (9.7C8  $\times$  S9), races carrying the factors aOL and AoL respectively. Normal asci with spores are formed in such matings, so that these experiments should be carried on before ascospores are discharged. A plate was inoculated on opposite sides with the two races 43 and T100. The mycelia soon met and produced perithecia. In order to avoid carrying over ascospores that might germinate, fragments of mycelium were taken from between points bearing perithecia. The fragments were thoroughly washed, and then placed on the agar in one arm of a U-tube. New perithecia soon appeared, and the mycelium grew around into the other arm where more perithecia and conidia developed. Conidia from the top of the agar slant in the second arm were sowed. Fifteen single conidia were isolated and grown in plate cultures as before. The next day one hundred 1-tip cultures representing the fifteen original conidia were made. At the end of ten days only one of these cultures showed perithecia. Twenty

of the sterile cultures were tested for their sex reaction. They all proved to be sex B like the parent no. 43. The results would suggest that unisexual hyphae like the parent 43 had split off from the bisexual mycelium soon after the latter had grown around into the second arm. Race 43 produces many more conidia than does T100 so that only one kind happened to be isolated. That nuclei of both sexes actually reached the second arm is proved by the fact that perithecia were formed there. The two kinds of mycelia might have grown around separately from the beginning, however, but this is not likely.

The experiment was repeated using the same parent races. This time of the twenty-three single conidia isolated thirteen were proved to be bisexual. A third test of the same kind was made again using races no. 43 and T100 as parents. Sixty-one 1-conidium isolates were obtained. Thirty-four of these were proved to be bisexual and twenty-seven unisexual. When grown on dextrose agar twenty-six of the unisexual isolates showed the bright orange-colored masses of conidia characteristic of parent no. 43. Only one isolate, no. C3, looked like the T100 parent which produces comparatively few conidia. This one was proved by test to be sex A, while the other twenty-six were sex B. These results prove again that bisexual conidia can be obtained by combining two unisexual races vegetatively.

Using the tester races S9 and S1 as parents U-tube experiments of the same sort were carried out. The two races were planted on opposite sides of a plate culture December 13. The mycelia had met across the center at the close of the third day. A bit of agar carrying anastomosing hyphal branches at the line of meeting was then transferred to a U-tube. The mycelium had grown around into the second arm by Dec. 19 and conidia as well as young perithecia were being developed. Thirty-five 1-conidium isolates were made from the conidia in the second arm. Twenty-nine of these were grown in tubes and six in plates. By Dec. 25, twenty-five of the tube cultures showed perithecia, the other four were still sterile. As soon as a few hyphal tips had grown out from the six plate transfers, twenty-three 1-tip isolates were made from the transplants which represented six different conidia. The results in this case showed that three of the original conidia were bisexual and three were unisexual.

In case of another experiment with the same parents, S1 and S9, twenty-eight 1-conidium isolates were obtained from conidia taken from the second arm. Only fourteen of these were bisexual. Since thirty-nine of the sixty-three single conidia originally isolated from the second arm of the U-tubes were proved to be bisexual, it is clear that by growing our unisexual tester races S9 and S1 together one is able to unite them vegetatively to give rise to a bisexual mycelium. The bisexual condition due to nuclear migration must have been fairly complete at the points from which the transplants were taken from the original plate culture.

In the course of this work over one hundred U-tube cultures of *N. tetrasperma* were studied. In every case but two, when perithecia matured in the first arm the mycelium that grew around into the second arm matured perithecia there also. There appeared to be a correlation between the number of fruit bodies developed in the two arms as though in some cases the heterokaryotic condition was more complete in some transplants than in others. Whether this represents a difference in rates of nuclear division or not is still a question.

Aronescu (1) incidental to her work on possible hormone action in sexual reproduction in case of *Neurospora sitophila* made eighty-six U-tube cultures in which she inoculated one arm of each tube in two places with conidia or bits of mycelium of opposite sex. In each case she found that while perithecia matured in the arm inoculated, only one kind of mycelium grew around into the other arm. No perithecia ever developed in the second arm. This would mean that unisexual mycelia of normally heterothallic races do not unite so readily to bring about a heterokaryotic condition and that the first mycelium to get started down the U-tube would be the only one to reach the other arm because it would use all of the oxygen available. Further work along the same line with both *N. sitophila* and *N. tetrasperma* will be necessary before the differences in behavior are understood. To make the tests comparable, parallel experiments must be made using, in one set, two kinds of conidia inoculated separately, and in the other allowing opportunity for hyphae of opposite sex to intermingle and use some of the mixture as inoculum.

*Neurospora sitophila*

One of the most interesting questions regarding the *Neurospora* species, as Lindegren (15) has pointed out, is the difference between those species like *N. sitophila* and *N. crassa* which are normally heterothallic and *N. tetrasperma* and *N. Toroi* which are normally bisexual and only facultatively heterothallic. Normal bisexual mycelia of the last two species form some unisexual conidia in culture and we have proved beyond question that unisexual mycelia derived from such conidia can be reunited readily by vegetative fusions to give the normal bisexual type of growth. The mechanism by which bisexual ascospores are cut out in these species and unisexual ascospores in case of *N. sitophila* is very effective. Is it merely the working out of the mechanism which determines the distribution of nuclei, or may there not be something more fundamental back of it, something which holds nuclei of opposite sex together in the same cells in *N. tetrasperma*, but keeps them apart in *N. sitophila* until a state of maturity is reached that demands their association in the phases of sexual reproduction? No one has heretofore described an experiment adequately proving that in *N. sitophila* or *N. crassa* bisexual mycelia can be obtained by vegetative fusions of unisexual hyphal branches or by fusions between germinating conidia or ascospores. As noted previously Köhler was not able to obtain such bisexual mycelia in this way. The following experiment would seem to confirm Köhler's report.

Races 56.2 and 56.6 of *N. sitophila* are non-conidial unisexual races. They were grown in plate cultures from opposite sides. Most of the perithecia were formed on the 56.6, sex A, side of the cultures. From each of eight different plates a small block of agar bearing a few immature but fertilized perithecia were transferred to separate plates. After new growth had developed about two centimeters in diameter, seventeen 1-tip isolates were made from each of the eight plates making in all 136 isolates. In similar experiments with *N. tetrasperma* and *Gelasinospora tetrasperma* reported in this paper every one of such isolate cultures produced perithecia. In exactly the same type of experiment with *N. sitophila* not a single 1-tip isolate produced perithecia with asci. This proves conclusively that in such a mating

heterokaryosis is certainly not readily brought about by vegetative fusions and nuclear migration. Moreover one can trace some hyphae from the 56.2 side well over on the 56.6 side of the plate, and some perithecia are usually formed on the 56.2 side also.

#### *Gelasinospora tetrasperma*

The writer is indebted to Dr. Dowding (Mrs. E. Silver Keeping) for cultures of her beautiful species *Gelasinospora tetrasperma*. The best possible proof that one can obtain a bisexual mycelium by the vegetative union of two unisexual races was obtained with this species. Our unisexual races Gel. 1 and Gel. 5 were grown from the opposite sides of a plate culture for two or three days. At the end of this time the mycelia had met at the center where hyphal fusions occurred abundantly. As soon as one could see that some perithecia were forming on the Gel. 5 side six small blocks of agar were cut out from various regions on that side of the line. These were transferred to individual plates. The hyphae were allowed to grow out so that single hyphal tips could be isolated readily. Every one of the thirty-eight 1-tip cultures so obtained matured perithecia with ascospores.

The same kind of test for nuclear migration was carried out using races of the unisexual Gel. 7 and Gel. 11 as parents. Here the perithecia always form on the Gel. 11 side of the plate. Four transplants taken from different parts of the area bearing young perithecia were made to separate plates. After new growth had become well established seven 1-tip isolates were obtained from new growth from each of the four original transplants. This time twenty-five of the 1-tip isolates produced perithecia abundantly, but three isolates from one transplant matured only a few fruit bodies at first, showing that heterokaryosis was not yet complete.

Later experiments proved that one need not wait until perithecia are formed before making the transplants. Nuclear migration following hyphal anastomoses here seems to take place rather rapidly. There is no evidence whatever in our cultures on 2 per cent Difco corn-meal agar plates that the mycelium from one side runs over on the other side; if it does one should obtain more unisexual 1-tip isolates. Neither microconidia nor

other asexual spores are formed in cultures of *Gelasinospora* according to Dowding, and such bodies were not seen in any of our cultures. When a unisexual clone is grown alone one can see what look to be long trichogynes emerging from ascogonia within the incipient perithecia. If a wort of young aerial hyphae from a clone of opposite sex reaction type should come in contact with these trichogynes the incipient perithecia would no doubt go on and mature.

#### ASCOCARP DISTRIBUTION PATTERN IN NEUROSPORA AND GELASINOSPORA

Lindegren (15) has proved conclusively that the genes for "pale" and "non-pale" and for the sex-reaction factors (+) and (-) are linked. Zickler (22) has also a very striking sex-linked character in a species of *Sordaria* which he calls *Bombardia lunata*. He finds that the factors for color of the ascospores in certain crosses are linked with the sex reaction factors. This enables him to study the first- and second-division segregations to great advantage.

More recently the writer (10) working on the effects of irradiation of ascospores of *Neurospora tetrasperma* and describing the effects of a recessive factor, l, lethal for ascospore delimitation when homozygous, pointed out that this same factor, l, seems to suppress the action of a factor O for bright orange-colored masses of conidia. It was suggested at that time, and it has since been proved more conclusively by Tai (21), that the factor O is linked with the sex-reaction factors.

#### *Neurospora tetrasperma*

The peculiar distribution of ascocarps in plate cultures of *N. tetrasperma* when one grows the unisexual component races from opposite sides of a plate culture (6, 8), referred to previously, p. 421, is such as to suggest the possibility of another linkage involving the sex-reaction factors. The writer has at various times isolated a number of unisexual clones by germinating the small ascospores. Mr. F. L. Tai and Dr. S. M. Pady working in our laboratory on other problems have turned over similar clones. When these clones are mated in plate cultures one finds a corre-

lation between the sex-reaction types and the manner of distribution of the perithecia. This is brought out rather easily in large tube cultures with long slants (PLATE 39, B). The top of the slant can be inoculated with mycelium or conidia of one sex and the bottom of the slant with the other.

Seven sex A clones were mated with three sex B clones in all possible combinations. The distribution pattern was very strikingly correlated with the reaction type, with one exception, fruit bodies forming on the sex A side. Clone S23 may represent a cross-over if there is a linkage. These cultures were kept several months but the patterns were not changed.

We have paired some twenty-one clones of sex A reaction with eighteen clones of sex B type in plate cultures. In some instances the distribution pattern was not absolutely fixed, but it was sufficiently constant to suggest that the distribution of perithecia in such matings may be genetic and possibly linked with the sex factors. There appear to be three types of distribution patterns. The first, figured in papers referred to previously, where the ascocarps were very definitely located on the sex A side of the meeting line with none of them on the B side. Then we had a few cases with the same but reverse pattern, the fruit bodies being well spread out on the sex B side with few or none on the sex A side. In the third series a few large perithecia were scattered down along the line of growth on the B side. Across the meeting line on the A side there was a region where the perithecia were so crowded as to make a black zone whose margin on the sex B side was very clearly marked at about the line of meeting, but where the margin on the sex A side was rather corrugated well out on the A mycelium as though nuclear migration was going in that direction but very slowly and completely as far as it goes (PLATE 40, B). It must be admitted that as between various combinations some interactions are more decided than others (PLATE 40, A). It is clear that while the distribution pattern has a genetic basis it is not determined solely by a sex difference or by a single pair of factors.

#### *Neurospora Toroi*

Our two unisexual strains, Toro A and Toro B of *Neurospora Toroi* Tai (21) grown together, originally produced most of the

fruit bodies on the A side. This species does not produce fertile hybrids readily with *N. tetrasperma*, but it does react very positively with it so that we can readily classify the races as to their sex reactions. The other interspecific reactions with this species in all cases agree, and the Chinese species *N. intermedia* Tai (21) does produce fertile hybrids with *N. Toroi*, so that we know that the clones one may isolate from the five species of *Neurospora* now being grown in culture all fall into one or the other of two sexual groups. When our albino non-conidial races 56.2 and 56.6 of *N. sitophila* are mated in plate cultures the most fruit bodies are generally found on the 56.6 side. It is very doubtful, however, that the distribution pattern regularly follows the reaction factors through such a wide range.

#### *Gelasinospora tetrasperma*

Reference was made previously (p. 421) to the very striking resemblance between *Neurospora tetrasperma* and *Gelasinospora tetrasperma* in the way the ascocarps are distributed over the cultures where heterothallic clones are mated. The writer isolated nine unisexual 1-ascospore clones from material generously sent him some time ago by Dr. Dowding. When these were mated in plate cultures in all possible combinations it was found that clones Gel. 4, -5, -9 and -11 were of one sex reaction and Gel. 1, -2, -3, -7 and -8 were of the opposite kind. With one exception the fruit bodies were very definitely distributed on the side of the clones in the first group (PLATE 39, A). If the distribution pattern is sex-linked, clone Gel. 3 may represent a cross-over spore because in the matings with this clone the fruit bodies were developed more or less on both sides of the line. The final distribution will be determined by the resultant effect of the opposing forces. If the progeny represented in the ascus in which Gel. 3 spore was formed had all been analyzed the reciprocal spore would have been obtained. If the distribution pattern is sex-linked, clone Gel. 3 should be mated against a cross-over to give a definite reverse pattern.

In another set of eight unisexual clones three cultures differed from the other five in their somewhat darker color and their fluffy white aerial hyphae and slower growth. With the idea

that the three were of the same sex and opposite to that of the others, they were grown separately against the other five. The results proved the correctness of the prediction. The line of meeting in each plate was well over on one side, but the fruit bodies in each case were distributed on the side of the more rapidly growing race. This proves that the direction in which the nuclei migrate the most readily is not necessarily correlated with the direction of growth of the most vigorous mycelium as has been observed in some other cases.

In such studies each clone should be tested against a particular tester strain because other genetic factors as well as unfavorable culture conditions must enter to disturb the distribution pattern. On 2% Difco corn-meal agar under the conditions of our cultures the pattern is rather constant and such as to suggest a linkage of some sort.

Colson (4, p. 217) says concerning the distribution of fruit bodies in cultures of *Neurospora tetrasperma*: "When inoculations of A and B are made on opposite sides of a petri dish, each strain grows completely across the medium so that the two strains become intimately mixed. As a result of this the perithecia do not form in a line at the meeting of the two mycelia but are scattered over the whole surface." The writer has not found any evidence for such behavior in his cultures of this species on corn-meal agar as we make it. If the nutrient medium contains too much starch or unfiltered corn-meal, or if one uses a 4% instead of our 2% agar medium then one might find the distribution pattern greatly altered because aerial hyphae then tend to grow out over the surface more generally. If water of condensation flows around in the plate, conidiation will affect the pattern. The linkage between the factors for bright orange-colored masses of conidia and sex-reaction factors is not very manifest on 2 per cent Difco corn-meal agar, but it is very striking in cultures on dextrose agar. So it is with the distribution pattern; to bring it out best one must have certain definite culture conditions.

Colson referring to first division versus second division segregation assumes that when a normal ascospore of *N. tetrasperma* taken from a four-spored ascus is proved to be unisexual this denotes a cross-over. Dodge, she says, never reported a cross-

over in his genetic studies. If isolating normal sized spores that are unisexual means finding a cross-over Lindegren and the writer have both reported such an event several times, and Kniep and Lindegren have diagrammed asci showing how this could occur. Second division segregation means a cross-over only when it is proved that in *N. tetrasperma* the spindle-fiber attachment points always reduce in the first division. Zickler, as noted previously, in order to explain his results has to assume otherwise in case of *Bombardia*. Nevertheless Colson's suggestion is a good one because if it is correct it gives us an additional means of analyzing linkages in these facultatively heterothallic species.

Zickler (22) claims to have what approaches one case of tetrapolar sexuality in *Bombardia*. His clones A lan. and a lan. are of opposite sex reaction. Since these clones produce very few incipient perithecia they are not only self-sterile, he says, but they are also cross-sterile. Clones a bulb. and A bulb. both produce incipient perithecia and also microconidia so that they are reciprocally cross-fertile. He finds that he can successfully "spermatize" clone a bulb. with clone A lan. but not clone A lan. with clone a bulb., and so with the other pair. He leaves one with the impression that while clones A lan. and a lan. can be used as spermatizers they can not be made to produce perithecia because they do not form ascogonia. We have not as yet been able to obtain these races from him for experimentation, so that it is impossible to say definitely what would happen if his races A lan. and a lan. were grown together in the same culture. There is the possibility, judging from what we find in similar cases in *Neurospora* and *Gelasinospora* (see also Aronescu, 1) that Zickler's races A lan. and a lan. are not cross-sterile as he leaves one to infer. Following hyphal anastomoses in a mixed culture incipient perithecia might very well arise and go on to maturity. In case of *Gelasinospora* where there are no microconidia spermatization is impossible, and we have furthermore proved that bisexual races readily result from anastomoses followed by nuclear migrations.

Lindegren (15) assumes that in nature where ascospores of *N. crassa* are discharged close together the mycelia arising from their germination immediately anastomose so that nuclei representing

different genotypes commingle in a coencytium which later becomes septate. Such a resultant mycelium being bisexual would, he says, produce perithecia were it not for sterility factors so abundant in these natural strains. He does not report having proved this by experiment and our experiment with *N. sitophila* reported above does not support this argument. He accepts the Moreau-Moruzi (17) hormone hypothesis without testing it out or without referring to work (27) disproving such an action. Zickler (22) has also proved the fallacy of this hormone idea. Stevens (19) claims to have induced self-sterile ascomycete mycelia to produce perithecia on exposure to ultra-violet light. Lindegren thinks that Stevens was working with strains which were bisexual heterokaryons. On the other hand, the writer does not question the correctness of Lindegren's view that in *Neurospora crassa* one might find in nature races that are heterokaryotic (temporarily?) containing nuclei of opposite sex, yet tending to remain self-sterile because of incompatibility factors. Such a condition in *N. tetrasperma* hybrid offspring had already been reported (8, p. 20-26), where races tended to remain sterile when grown alone, but when mated separately with the tester strains of opposite sex produced perithecia in both matings. If one did not know the nuclear story he would insist that the tester strains were not unisexual but were merely self-incompatible and cross-compatible in their reactions. Similar incompatibilities often exist in *Gelasinospora*. Our unisexual race Gel. 13 is very fertile with races Gel. 15 and Gel. 17, but is very incompatible with Gel. 18 and Gel. 19; in fact, so far it is rather cross-sterile with these two although proved beyond question to be of opposite sex reaction.

Further experiments with *N. sitophila* to be reported in another connection show that this normally heterothallic species is fundamentally very different from those facultatively heterothallic species like *N. tetrasperma* and *Gelasinospora tetrasperma*.

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## EXPLANATION OF PLATES

Photographs mounted to show original orientation unless stated otherwise.

## PLATE 37

*Neurospora sitophila* race 56.2 grown alone: A, section of incipient peritheium showing the trichogyne-like receptive hypha from the base of the ascogonium; B, trichogyne tip end above and outside the young peritheium, the branched basal part of ascogonium in lower part; C, D, two adjacent sections of two incipient perithecia; at the right in C, a receptive hypha, the only one emerging from this perithecial fundament; the series of sections of the peritheium at the left show no emerging receptive hyphae; E, trichogyne growing around in the sterile tissue seeking a way out; F, a trichogyne emerging at the left and a central cell of the ascogonium sending out another receptive hypha; G, trichogyne pushing in between sterile cells on its way to the surface; H, tip end of ascogonium here gave rise to two trichogynes, only one showing in this section; the subterminal cell also branched (see plate 38, F, for enlarged view of same section); I, section showing sterile trichogyne-like hairs arising from the outer cells of the incipient peritheium, having no connection with the ascogonium and can not be receptive; J, central cell of ascogonium giving rise to a trichogynous element, not common.

## PLATE 38

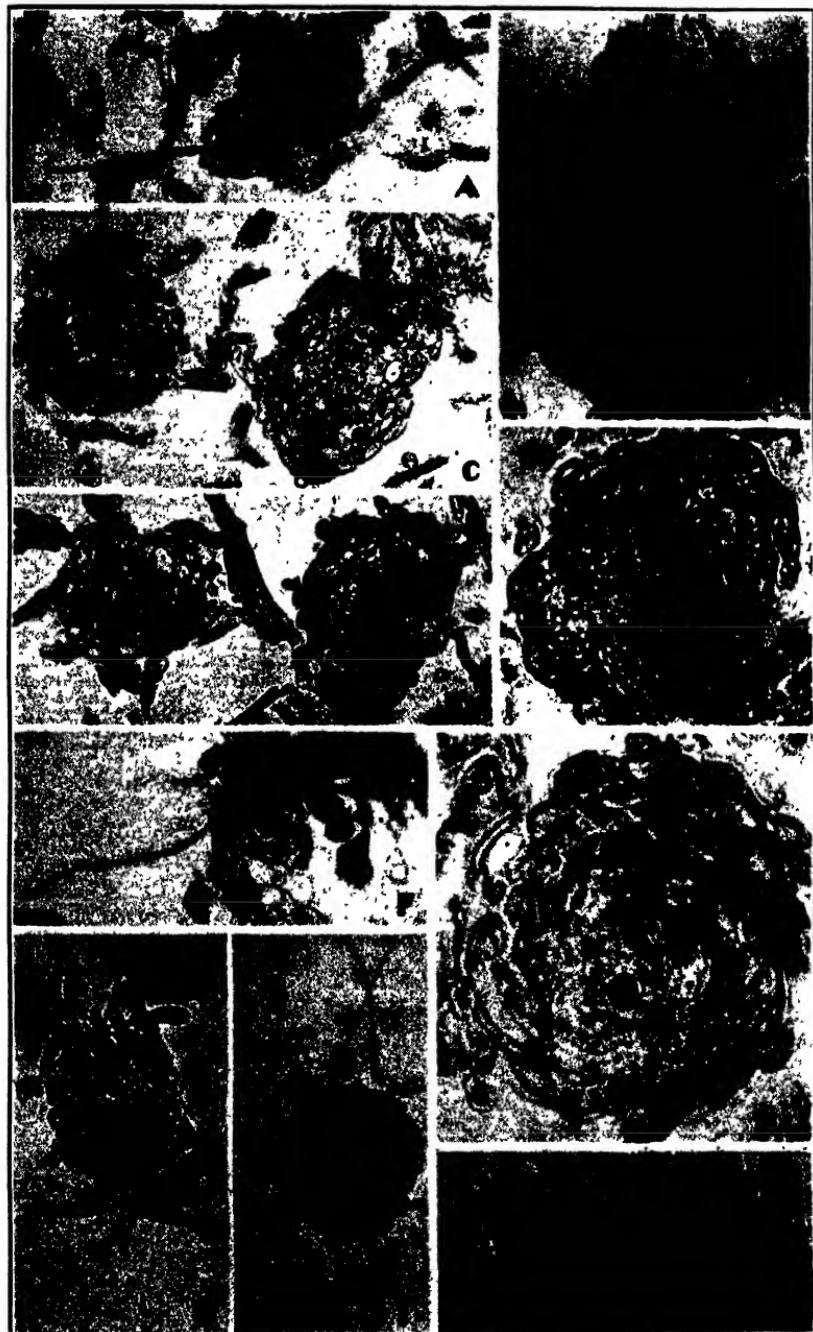
*Neurospora sitophila*: A, trichogyne-like receptive hypha, probably arising from a basal cell of the ascogonium; B, hypha extending out through the base of the peritheciun is not a trichogyne, but may well have been receptive; C, branched receptive hypha from basal cell of ascogonium, next section showed the connection between this branch and the rest of the ascogonium; D, photograph, inverted, of receptive hypha from base of ascogonium, no trichogyne from apical end; E, basal cell branched to give rise to two receptive hyphae, the ascogonium branched again above (see H for the next section of this incipient peritheciun, photograph mounted at 90°, shows either another branch from the same basal cell or the other half one of those shown in E); F, more highly magnified section of same body shown in plate 37, H; G, open cytoplasmic connection between basal cell of ascogonium and the branched receptive hypha which was early surrounded by sterile tissue, no trichogyne found in the series of sections; H, see legend for E; I, coiled ascogonium showing basal cell branched, incipient perithecia often inverted.

## PLATE 39

A, plate culture of *Gelasinospora tetrasperma* showing perithecial distribution pattern which is exactly like that illustrated by Dowding (13) and is the same type of pattern one obtains in many matings of *Neurospora tetrasperma* referred to in our text (p. 421); B, tube cultures of *Neurospora tetrasperma* in which the top of the slant was inoculated with a sex A strain in each case; the number at the left on each tube refers to the sex A race, and the one at the right to the particular sex B race used; perithecia mostly formed on the sex A mycelia. (These cultures were kept several months without any material change in the pattern except the addition of numbers of perithecia down in the agar but always on the A mycelium.)

## PLATE 40

*A*, plate culture showing odd distribution pattern in which nuclear migration was not uniform; the streaks of perithecia down along lines of growth showing where nuclei from S33, which is sex B in reaction, moved down along the S22, sex A, hyphae; *B*, S4 is sex A, and S27 is sex B in reaction; the first perithecia formed were those scattered on the S27 side; the dense zone of ascocarps showing that heterokaryosis was very complete in this region, nuclear migration from S27 down S22 hyphae very slow. Nuclear migration toward the S27 side also.



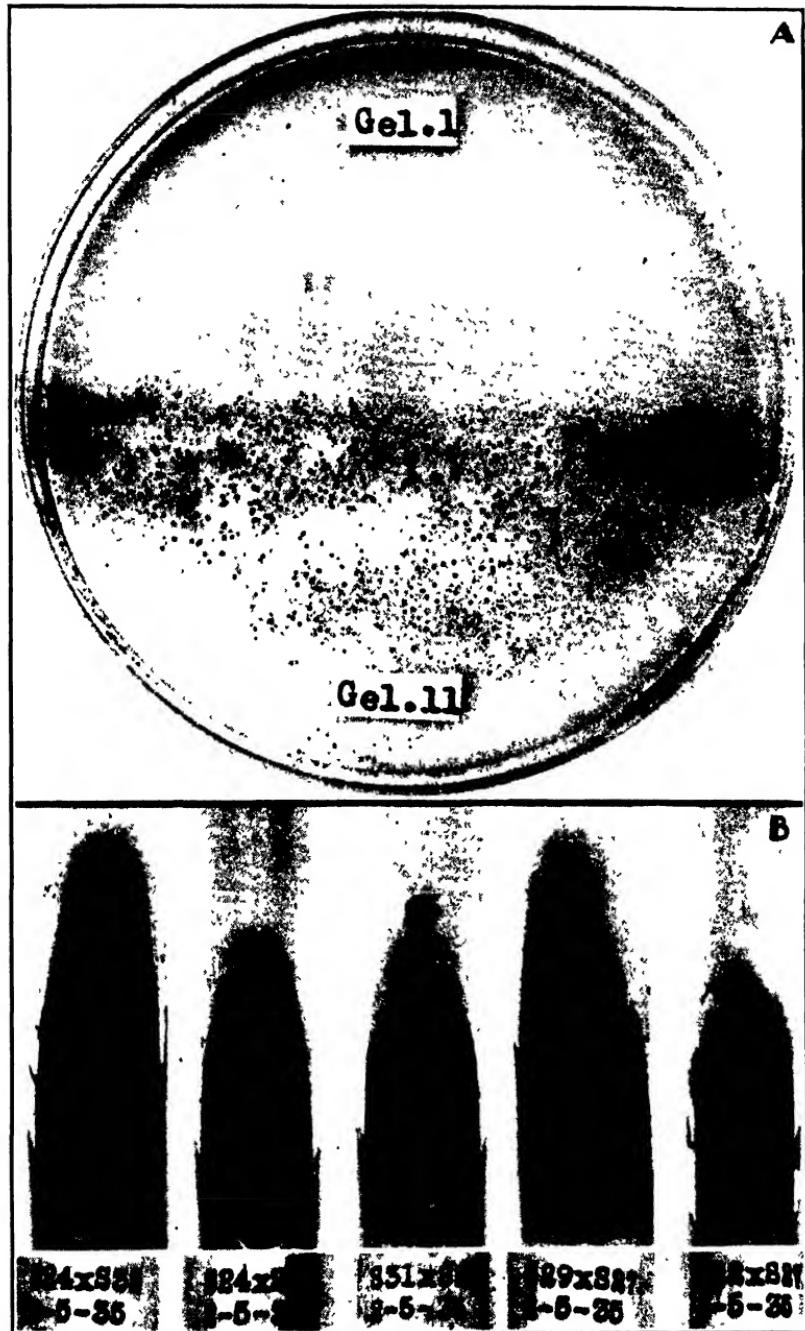
INCIPIENT PERITHECIA OF NEUROSPORA





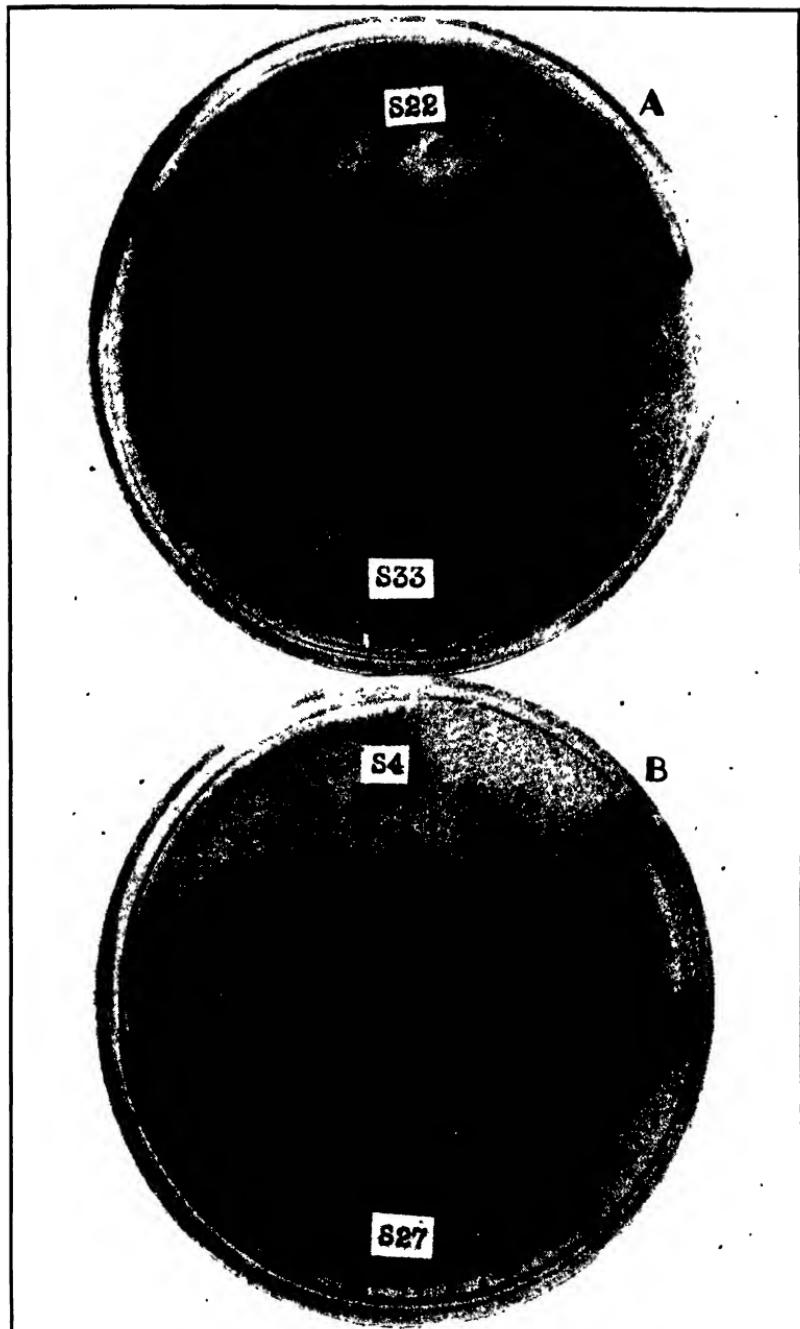
INCIPIENT PERITHECIA OF NEUROSPORA



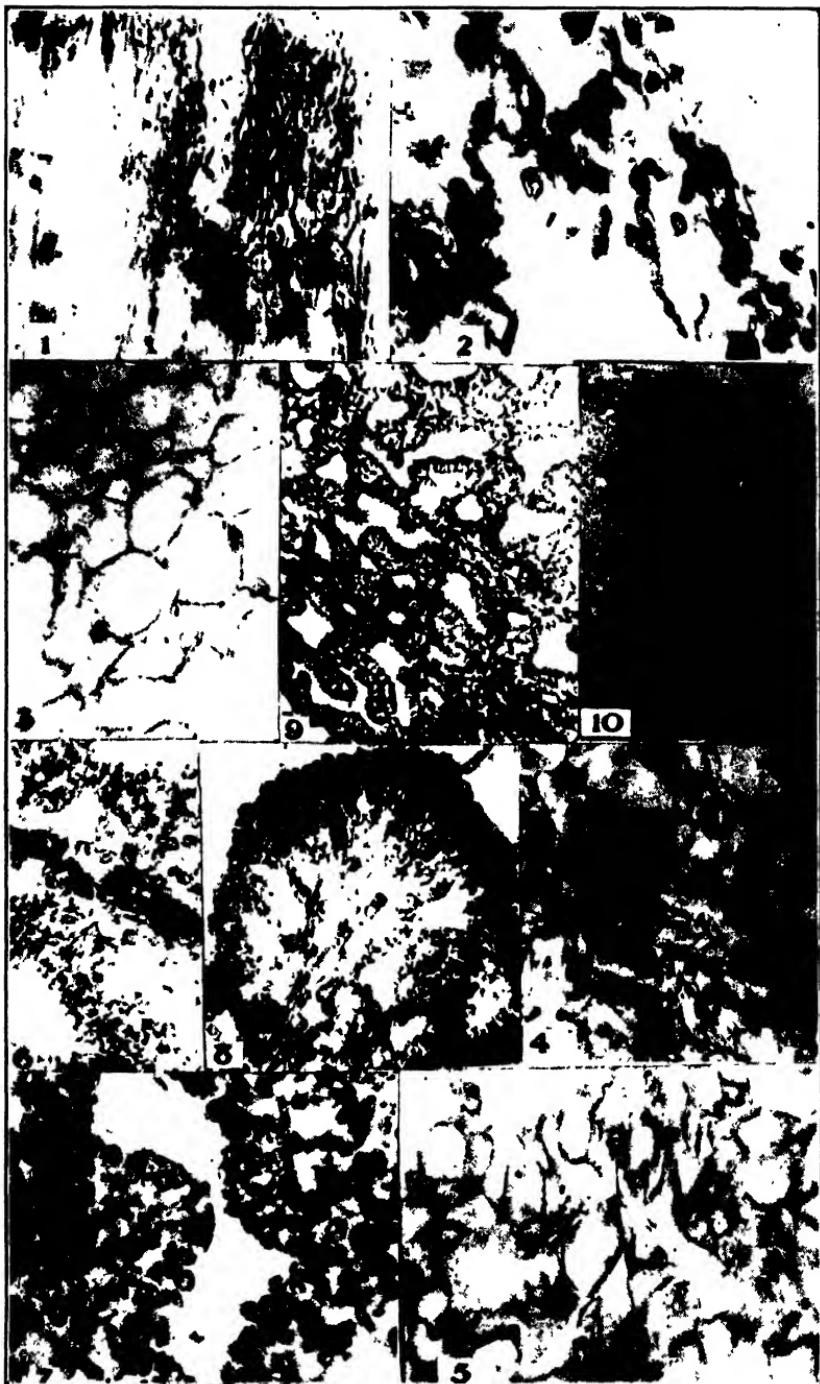


DISTRIBUTION PATTERNS OF GELASINOSPORA AND NEUROSPORA





DISTRIBUTION PATTERNS OF NEUROSPORA



Figs. 1-10. *Calvatia craniiformis*.

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## THE DEVELOPMENT OF CALVATIA CRANIIFORMIS

DELBERT SWARTZ<sup>1</sup>

(WITH 10 TEXT FIGURES)

It has been repeatedly pointed out by various workers (1, 2, 3, 4, 8, 13, 14), that the taxonomic arrangement of species of fungi should rest firmly upon facts gained from a study of the detailed development of as many species as possible. In previous papers the writer (13, 14) has reviewed the literature dealing with studies of the development of species of Lycoperdaceae. It is hoped that the following report of studies of *Calvatia craniiformis* (Schw.) Fries may add something of value to our knowledge of relationships in the Lycoperdaceae.

### MATERIAL AND METHODS

In October, 1933 the writer obtained a complete developmental series of fruit bodies of this species. The material was killed in Flemming's weaker solution and imbedded in paraffin in the usual way. For the younger stages entire fruit bodies were used; in the larger fruit bodies sections of various parts were imbedded. Sections were cut 7  $\mu$  thick, and stained in Heidenhain's iron-alum haematoxylin.

<sup>1</sup> Research paper, Journal Series, University of Arkansas, No. 363.

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## OBSERVATIONS

*Description of fruit body:* Fruit body very large, obovoid or turbinate; base stalk-like, thick, stout with a cord-like root; peridium thin, papery, smooth, pallid or grayish, breaking up into uneven areas at maturity and falling away. Subgleba honeycombed, spongy, concave at top, and long time persistent. Gleba at first white, becoming greenish yellow to ochraceous at maturity. Capillital threads slightly branched, sparsely septate, about as thick as the spores. Spores spherical, smooth with a short pedicel 2.8–4  $\mu$  in diameter.

*Rhizomorph:* The rhizomorphs of *Calvatia craniiformis* are well developed, and for the most part are similar in structure to those of other species of Lycoperdaceae (13). In longitudinal section there are three layers: (1) Outer cortex layer, (2) Subcortical layer, (3) Central core. The outer cortex layer is composed of relatively thick, blunt, interwoven hyphae which stain more deeply than do the hyphae of the other layers. They arise from the more compact subcortical layer, but in contrast to them they have no visible internal differentiation. This seems peculiar because the hyphae of the outer layer are connected to living hyphae of the layer just beneath. At the tip of the rhizomorph this cortex makes up the greater part of the total diameter.

The subcortical layer is very thin and is composed of hyphae which for the most part are parallel to the long axis. There is comparatively little or no interweaving. These hyphae have thin walls and do not stain deeply. On the inside they are continuous with the larger hyphae of the central core.

The component hyphae of the central core are usually several times as thick as those of the subcortex. Septa occur frequently. There are not as many large deeply staining crystals as have been seen in other species (13, 14). Hyphae of the subcortical layer may enter into this central core, continue in the larger size and shape, and then enter once more into the subcortex where they assume the size characteristic of the hyphae of that region. The most conspicuous feature of the rhizomorph is the extremely large size of the hyphae of the central core; *Lycoperdon pedicellatum* Peck (14) is the only investigated species in which the size is comparable.

When a rhizomorph of *Calvatia craniiformis* branches, the resulting branch arises chiefly from the hyphae of the central core and not from the hyphae of the subcortex (FIG. 1).

*Formation of the fruit body:* The fruit body arises from the tip of a rhizomorph or from a lateral branch of it. This species is similar in this respect to other investigated Lycoperdaceae. Young fruit bodies are surrounded by hyphae resembling those of the cortex of the rhizomorph, but the homogeneous interior is made up of hyphal branches originating in the central core.

*Peridium:* The peridium in this species is similar to the same structure in *Calvatia saccata* (13). There is no division into the exoperidium and the endoperidium; instead this outer layer becomes pseudoparenchymatous at an early age. The hyphae do not become arranged radially parallel as in other species. This arrangement is brought about by the interweaving of the tangential hyphae, and is somewhat similar to the layer of pseudoparenchyma in *Bovista plumbea* Pers. (13). The development of this layer is more complete in *Calvatia craniiformis* than in any other species (FIG. 2, 3, 4). On the periphery there are a few loosely scattered tangential hyphae; they are present in greater numbers at first, but seem to wear away gradually. In the younger fruit bodies these hyphae are simply extensions of the cortical layer of the rhizomorph, while in the more mature fruit bodies they are the outermost extensions of those of the pseudoparenchyma. This is further reminiscent of *Bovista plumbea* but as will be shown the two layers are really quite different. Between the pseudoparenchyma (FIG. 3) and the gleba is a region of somewhat elongated flattened cells (FIG. 4, 5). These cells are not differentiated into any structure which can be compared to the endoperidia of other species; instead it simply marks the extension of the hyphae of the gleba into the pseudoparenchyma. There is no separate layer formed; the hyphae are connective. The flattening is due to pressure on the inside due to the rapidly expanding gleba.

*Gleba:* The gleba is formed in the same general way in *Calvatia craniiformis* as in other investigated species (13). In very young fruit bodies it is composed of a homogeneous mass of hyphae which are continuous with and very similar to the hyphae of the central core of the rhizomorph. As growth continues these hy-

phae become more closely compressed because of the extremely active branching of the original threads. Although the fruit bodies are ordinarily quite large when mature, glebal differentiation begins at a very early age. Cavities are formed and become lined with their characteristic even hymenium when the fruit bodies are quite small. A few scattered spores have been seen soon after the first hymenial layers have been laid down. This has been reported in other species (12, 13). The presence of spores at such an early age is significant; it simply indicates that the stage of glebal development can not be correlated with the size of the specimen in question. These results differ from some reports of other investigators (9). In addition to the hymenial layer, there is a tramal layer and a subhymenial layer found in developing fruit bodies (FIG. 6). The trama is composed of large, septate, multi-nucleate hyphae which are rather closely compressed and form a very pronounced strand which winds in and out between the rapidly forming cavities (FIG. 6). The tramal hyphae branch freely on either side to form subhymenial layers. These layers of subhymenia are rather poorly developed in this species, and when present appear rather late. When they are absent the basidia arise directly from the trama (FIG. 7).

Additional cavities are formed rapidly in young fruit bodies by a mechanical separation of the hyphae of the trama. They enlarge in two ways:—(1) by the insertion of new basidia and (2) by the coalescing of neighboring cavities (FIG. 6, 7). Frequently large, conspicuous hyphae arise in the trama, pass through the hymenial layer, through the cavity, and are lost in the hymenial layer on the opposite side of the cavity. Such threads are evidently tramal hyphae which have not been pushed apart during the formation of the cavity, and are often seen in various stages of disintegration in fruit bodies of more mature age. The gleba is always more completely formed in the apical portion, and matures there first. The spores are attached to the basidia by sterigmata and are first visible as terminal swellings on them.

At the base the gleba is joined to the sterile base (FIG. 9). Ordinarily the hyphae at the region of connection become rearranged forming a horizontal layer. This rearrangement permits the complete wearing away of the spores and capillitium without dis-

turbing the sterile base. It is suggestive of the columella described by some workers (12) in other species.

The details of nuclear behavior were not studied.

*Capillitium:* The capillitium in *Calvatia craniiformis* is formed in the same way as described for related species (12, 13, 14). In this species the first formed capillitium is visible near the connection of the peridium and the gleba, but later it is of general occurrence throughout the trama.

*Sterile base:* The very well developed sterile base appears some time after the differentiation of the gleba has begun. In my specimens the hyphae of the sterile base are not so well organized as in certain other species (13). They are at first loosely interwoven and gradually assume the arrangement reported in similar structures in related species (13). As was pointed out above the sterile base is separated from the gleba by a fairly well organized layer of hyphae (FIG. 9). When the fungus has matured and begins to dry out this layer evidently becomes completely formed; it is not unusual to see sterile bases still attached to the ground long after the glebal mass has completely disappeared.

*Dehiscence:* The peridium gradually dries and falls away in flakes, thereby exposing the mature spore mass. Sometimes the entire peridium is broken up before any of it falls away. Just why the peridium should break up as it does is not clear; there are no indications of the formation of weakened hyphal zones.

#### DISCUSSION

The results of this study of *Calvatia craniiformis* show that the developmental processes proceed along the same general lines previously reported for other investigated Lycoperdaceae. The additional findings indicate once more that related species have their own characteristic mode of development. These developmental differences, as might be expected, indicate hitherto unsuspected relationships.

The peridium which has been ordinarily considered two-layered in the Lycoperdaceae, here, as in the case of *Calvatia saccata* (Fries) Lloyd, is shown to be composed of only one well-defined layer. A thin layer of adhering cortex hyphae and the ends of

some of the peridial hyphae make up the periphery of the peridium (FIG. 10). There is no complete, permanent extension of this layer around the upper globose part of the mature fruit body; hence it cannot be considered as a separately differentiated peridial layer. The differences that appear in the hyphae of the peridium proper are due to external changes resulting from internal pressure caused by the expansion of the gleba. Naturally this layer, subjected to pressure from the inside only, shows the results of this by a somewhat looser arrangement at the periphery (FIG. 5). This outer layer is connected directly to the region just beneath where the cells have been subjected to additional pressure from the inside and have become definitely pseudoparenchymatous; they have been limited on the outside by the cells already there. Further this pseudoparenchyma is connected to hyphae which arise in the gleba (FIG. 4); they are not tangential in direction, not compactly compressed, nor do they form a definite layer which in any way compares to the endoperidium of the lycoperdons. This outer covering, the peridium (FIG. 10), is interpreted as a one-layered structure in which the cells at various distances from the periphery have become somewhat modified because of various growth tensions. In certain descriptions of the calvatias (5, 10) mention has been made of two layers; it seems interesting to note that the two investigated calvatias, *Calvatia saccata* and *Calvatia craniiformis*, show the same general make-up of the peridium. In the light of this evidence it seems safe to consider a one-layered peridium characteristic of the genus. It will be interesting to see just how general this condition is among the remaining uninvestigated species.

The peridium of *Calvatia craniiformis* shows still another somewhat unsuspected relationship. The outermost fringe of hyphae in *Calvatia craniiformis*, as well as the layer of pseudoparenchyma, is very similar in appearance to the two outermost layers found in *Bovista plumbea*. Sculpturing is not found on the exterior in either species. However, the development of this outermost layer of tangential hyphae in *Bovista* indicates a relationship of this species to *Geaster* (13). In *Calvatia saccata* the outermost region of the peridium is composed of adhering meal-like granules, and

these were considered the remains of any exoperidium it may have had (13). In *Calvatia craniiformis* the loosely arranged external layer may be interpreted in the same manner. The spines characteristic of the outer peridial layers in *Lycoperdon pulcherrimum* Berk. and Curt., *Lycoperdon Wrightii* Berk. and *Lycoperdon pyriforme* (Schaeff.) Pers. have been considered homologous with the outer tangential region in the very well-developed peridium of *Bovista plumbea*; there seems to be nothing in either of our calvatiæ which can be considered homologous to these structures. This opinion is held in spite of the loosely adhering granules in the one and the tangential hyphae in the other (FIG. 10). It is evident that these are simply related to a small part of the exoperidium of the others.

The gleba in this species, during its differentiation and subsequent development is essentially the same as in the other investigated species of Lycoperdaceæ. Here, as elsewhere, the hymenium arises from the trama (FIG. 8). The trama of this species as in *Calvatia saccata* is well developed (FIG. 6). The subhymenial layer, however, is poorly developed; this is similar to the condition found in *Lycoperdon Wrightii* and *Lycoperdon pulcherrimum*. However, in *Calvatia saccata* the subhymenium is well developed. In some specimens of *Calvatia craniiformis* the hymenium branches directly from the trama; in others a definite subhymenium is formed. This unexplained variability can not be relied upon to furnish conclusive evidences of relationship or lack of it.

Cavity formation, as well as spore formation, is similar to the other investigated Lycoperdaceæ. Capillitium is formed in this species from living hyphae and seems to function in the disposal of waste material. The only difference is in the appearance of the first threads in the region of the connection of the gleba and peridium; this has not been observed by the writer in other species. It has, however, been reported by Cunningham (6) and by Rehsteiner (12). This condition is only temporary because the capillitium threads appear in greater numbers in the region of the trama as the fruit body undergoes the later stages in its development. Aside from these facts the structure of the gleba is similar to other species.

The rearrangement of the hyphae at the region of the union of the gleba and the sterile base is somewhat similar to that observed by the writer (14) in *Lycoperdon pedicellatum* Peck and by Miss Rabinowitsch (11) in *Lycoperdon depressum* Bon. However, it scarcely seems plausible to consider this line of separation as a sufficiently differentiated structure to be designated a columella. On the other hand, if we are to consider such structures columellae regardless of how well or how poorly they are developed, then certainly *Calvatia craniiformis* has a columella. Such an interpretation seems undesirable; it seems that the sense of 'grenz-linie' as used by Miss Rabinowitsch is more satisfactory.

The sterile base of *Calvatia craniiformis* comprises about half of the entire fruit body at maturity. The well developed sterile base is suggestive of a relationship to the majority of the lycoperdons. However, this characteristic seems to vary within the genus *Calvatia*. *Calvatia gigantea* Batsch. has comparatively little sterile tissue in proportion to its size. In *Calvatia saccata*, *Calvatia cyathiforme* Bosc. as well as in *Calvatia caelata* Bull. the basal portions are very well developed. This character can hardly be considered of importance in establishing relationships unless it can be shown that *Calvatia gigantea* does have a sterile base sometime during its development.

The method of spore dispersal in our species is typical of the known calvatias. It differs somewhat from the condition described for *Calvatia saccata* which at times has an apical opening similar to the lycoperdons. This condition has not been observed in *Calvatia craniiformis*. Although the peridial hyphae become rearranged at the time of pore formation in the lycoperdons and the geasters, no such changes have been seen to account for the uneven breaking up of the peridium of the calvatias.

The large rhizomorph of this species is similar to the other investigated species and differs only in the poor development of the subcortical layer and the extremely large hyphae of the central core. These characters do not seem significant.

The above observations indicate that additional studies of the development of other species of *Calvatia* will prove of value and

will aid in the ultimate understanding of relationships in the Lycoperdaceae.

The writer wishes to express his thanks to Dr. D. M. Moore for the collection of this material.

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#### DESCRIPTION OF FIGURES

Fig. 1, a branching rhizomorph (Note the large hyphae of the central core at base; also the three characteristic layers of the rhizomorph); 2, the

outermost tangential hyphae of the periphery of the fruit body; 3, extremely well developed pseudoparenchyma of the central part of the peridium; 4, the somewhat elongated, flattened hyphae connecting the gleba and the peridium; 5, the looser pseudoparenchyma of the peridium; 6, the well developed trama layer between the cavities; 7, showing basidia arising directly from the trama; the coalescing of neighboring cavities; also a few sterigmata on the basidia; 8, the details of the hymenium and its relationship to the branches of the trama; 9, the region of union of the fertile gleba and sterile base; also the rearrangement of hyphae at the same point; 10, photograph of the entire peridium and the adjoining gleba; note the pseudoparenchymatous condition of the peridium.

# SOME MISCELLANEOUS FUNGI OF THE PACIFIC NORTHWEST<sup>1</sup>

S. M. ZELLER

(WITH 3 TEXT FIGURES)

This paper reports one genus and four species as new, two new combinations, four species new to America, and thirty-six new to Oregon. The types of the new species are deposited in the Herbarium of the Department of Botany, Oregon State College. Unless otherwise stated the localities referred to are in the state of Oregon. Ridgway's standard was used for describing colors.

## PHYCOMYCETES

### 1. DICRANOPHORA FULVA Schr.

Parasitizing *Boletus*, Benton, Linn, and Jackson Counties. Common in October. Perhaps this is the first report of this fungus from western United States. Thaxter, however, found it parasitic on *Boletus* in Maine.

## ASCOMYCETES

### 2. VALSA CORONATA Fries.

On twigs of *Castanopsis chrysophylla*, near Eugene, Lane County. December.

### 3. PORONIA OEDIPUS Mont.

On horse dung, Corvallis. April.

### 4. Leptosphaerulina Sidalceae sp. nov.

Peritheciis nigris, oblato-sphaeroideis, subepidermicis erumpentibus, 300-400  $\mu$  crasso, 200-250  $\mu$  alto, ostiolo papillato; ascis octosporis cylindraceis, in pedicellum brevem attenuatis, 140-180  $\times$  30-37  $\mu$ , apophysatis; sporidiis

<sup>1</sup> Published as Technical Paper No. 213 with the approval of the Director of the Oregon State Agricultural Experiment Station, Corvallis. Contribution from the Department of Botany.

distichis, ellipsoideis utrinque obtusulis, muriformiis 6- vel 7-transeptatis, fulgineis,  $37-50 \times 14-23 \mu$ .

In caulis emortuis *Sidalcea campestris*, Benton county, Oregon, Amer. bor. Aprilis.



FIG. 1. Canker of *Alnus rubra* caused by *Nectria ditissima* var. *major* Wr.

*Perithecia* oblate-sphaeroid, black, superepidermal, erumpent,  $300-400 \mu$  broad,  $200-250 \mu$  deep; ostiole papillate; asci 8-spored cylindrical, short-stipitate,  $140-180 \times 30-37 \mu$ , aparaphysate; spores muriform with 6 to 7 transverse septa, fuliginous,  $37-50 \times 14-23 \mu$ , broadly ellipsoid with blunt ends, usually 2-seriate.

On dead flowering stems of *Sidalcea campestris* Greene, North Benton County. April.

**5. NECTRIA DITISSIMA** Tul. VAR. MAJOR Wollenweber.

On *Alnus oregona* near the summit of the coast range on the McMinnville-Tillamook highway. Tillamook County. March. Collected by L. N. Gooodding.

As the illustration (FIG. 1) shows, this fungus causes a canker of alder quite typical of the *Nectria* cankers of other hosts. So far as the writer knows, this is the first report of this form in North America, it having previously been found in Norway only. Ours is typical with ascospores  $17.7-22 \times 7-9.2 \mu$  and the characteristic asci which have about 4 or 5 spores crowded near the upper end and 3 or 4 in a row below, as illustrated by Richter<sup>2</sup> and Wollenweber.<sup>3</sup>

**6. HYSTEROGRAPHIUM FRAXINI** (Pers.) De Not.

On *Fraxinus oregona*, Eugene-Lorraine Road. Lane County. March.

**7. COCCOMYCES DENTATUS** (Kunze & Schm.) Sacc. VAR. HEXAGONUS Penx. & Sacc.

On leaves of *Mahonia nervosa*, Alsea Mt., Benton County. March.

This collection is upon a different host than before reported but it answers the description for the above species and variety. The perithecia are nearly all hexagonal, epiphyllous and hypophyllous, shiny black, 1 mm. in diameter on nearly white spots. The asci are  $90-100 \times 7-9 \mu$ , and the spores are linear to acicular, continuous,  $27-36 \times 1-2 \mu$ .

**8. ATROPELLIS PINICOLA** Zeller & Gooodding.

On living branches of *Pinus lambertiana*, Dorrington, Calaveras County, California, Aug. 16, 1930 (Frank A. Patty) and on *P. Strobus* in a planting, Mt. Hebo, Tillamook County, Oregon, Aug. 19, 1931 (L. N. Gooodding) and July 23, 1932 (L. N. Gooodding and Geo. Root).

<sup>2</sup> Richter, H. Die wichtigsten holzbewohnenden Nectriën aus der Gruppe der Krebsreger. Zeitsch. Parasitenk. 1: 24-75. illus. 1928. (See p. 48.)

<sup>3</sup> Wollenweber, H. W. Pyrenomyceten. Studien II. Angew. Bot. 8: 168-212. illus. 1926. (See Taf. IV, 32.)

*Pinus Strobus* is a new host for this parasite and the collection on *P. lambertiana* extends the southern known range of the disease.

9. **CENANGIUM PINIPHILUM** Weir.

Parasitic on *Pinus albicaulis*, Taylor Burn, Lane County. Sept. 3, 1931 (*Conrad Wessela* and *L. N. Goodding*). This is a new host for this fungus.

10. **CIBORIA AMENTACEA** (Balb.) Fuckel.

On fallen male catkins of *Alnus rubra* Waldport. March.

11. **BELONIOSCYPHA CILIATOSPORA** (Fuckel) Rehm.

On stems of *Aster* (*A. novi-belgii* x *A. Douglasii*) which have been dead about a year. December.

12. **PERROTIA FLAMMEA** (Alb. & Schw.) Boud.

On acorns and cups of *Quercus garryana*, near Corvallis. May.

This species has previously been reported west to Montana and Colorado.

13. **RHIZINA INFFLATA** (Schaff.) Karsten.

Associated with and parasitic on roots of *Pinus contorta* in sandy hummocks near the ocean beach at Big Creek, Lincoln County. There were great quantities of the fruiting bodies on the sand covering an area about 30 feet in diameter. If this fungus is not parasitic it at least smothers seedlings 2 to 3 years old. This is the first report of this species west of Idaho.

14. **LEOTIA LUBRICA** Fries.

In heavy moss under conifers, Coos County. November.

15. **MORCHELLA BISPORA** Sorokin.

On the ground in mixed woods, near Peoria, Linn County, and Jefferson, Marion County. April.

These are trim, erect plants with nearly totally free caps. The asci are 2-spored, the spores measuring  $74 \times 18.5 \mu$ . This species has previously been reported as far west as Minnesota.

**16. DURANDIOMYCES PHILLIPSII (Mass.) Seaver.**

On old burned straw stack, Tygh Ridge, 15 miles south of Dufur, Wasco County. November. Collected by Dr. Roderick Sprague.

**17. ASCOPHANUS TESTACEUS Phillips.**

Growing in a damp chamber on paper and sclerotia from lettuce which had been collected in Benton County. May.

**BASIDIOMYCETES****18. GYMNOSPQRANGIUM NELSONI Arthur.**

On *Juniperus occidentalis* Nutt., Cove. Union County. April. Small galls on the branches are associated with the telia of this rust.

**19. TRIPHRAGMIUM CLAVELLOSUM Berk.**

On leaves of *Aralia racemosa* L. (American spikenard or Indian-root), Yoncella, Douglas County. July.

This is the first report of this rust from the Pacific Northwest.

**20. Clavaria brunnea sp. nov.**

Solitaria, 11-12 cm. alta, 7-8 cm. crassa; stipite distincto, profundo radicans, 1-2.5 cm. crasso, pallide brunneolo, basi pallide cremeo; rami plures, errecti, plerumque recti, cylindrici, interdum furcati, apicibus briviter 2-3-fidis, angulis acutis, superficie pulverulentis, "pecan brown" vel "fawn color," "army brown" vel "natal brown"; carne cremea vel flavescente, amarescente; sporis pallide brunneis, oblongis vel ellipsoideis, subverrucosis, 8-10 X 4-5  $\mu$ .

Ad terram inter folia dejecta praecipue Coniferarum, prope Seal Rocks, Lincoln County, Oregon, Amer. bor.

Among fallen leaves, especially of conifers, near Seal Rocks, Lincoln County. October 7, 1931. Collected by Mrs. Frank York.

*Plants* solitary, 11-12 cm. tall, 7-8 cm. broad, with a distinct, rather deeply rooted stem, 1-2.5 cm. thick; *branches* numerous erect, mostly straight, pencil-like, sometimes branched, tips usually short-forked, angles sharply acute; surface pulverulent; color of branches "pecan brown" to "fawn color" in lighter shades but "army brown" to "natal brown" where darker, drying "Buck-

thorn brown" ("mummy brown" where bruised), tips concolorous and stem lighter brown to almost creamy white in parts; *flesh* creamy to flavescent, slightly bitterish; *spores* light brown, oblong to ellipsoid, slightly roughened,  $8-10 \times 4-5 \mu$ .



FIG. 2. *Clavaria brunnea*.

This species reminds one of *C. grandis* in color and size (FIG. 2) but it differs in size and shape of spores, the acute angles of the branches and it occurs in coniferous woods. The spores are merely

roughened while in *C. grandis* they are distinctly echinulate or verrucose.

## 21. CLAVARIA SANGUINEA Pers.

In coniferous woods, Lincoln County. November. Frequent.

This beautiful red *Clavaria* was previously reported by Coker from Massachusetts and North Carolina. The Oregon plants when fresh are pink to red throughout except the whitish base. These colors are mostly spinel pink to spinel red or alizarine pink on shaded sides.

## 22. CLAVARIA STRICTA Pers.

On alder wood from white rhizomorphs, Alsea Mt., Benton County. November. Frequent.

*C. stricta* has been found and recognized several times in recent years. This is the first report of the species from the Pacific slope. Coker, however, reported it from Idaho.

## 23. BOLETUS EASTWOODIAE (Murrill) Sacc & Trott.

Solitary, in clay soil under *Quercus garryana*, in the Starr Creek district, Benton County. October. (FIG. 3.)

Two specimens were found, one in fine condition but the other was badly decayed. Measurements of the older specimen were taken. I have referred the collection to *Boletus Eastwoodiae* because it agrees in the outstanding characters but differs in the shades of color. This may be due to the fact that the original description was prepared from dried specimens. I have not seen the type. The following descriptive notes are presented to augment the original description.

*Plants* solitary; *pileus* up to 18 cm. broad, thick, solid, surface soft glabrous, shining to innately-reticulate-areolate, pinkish buff to light olivaceous shades in the areolae, with pink shades as on the stipe; *context* white to yellowish, changing to blue when wounded or cut, but soon returning to the original color; *tubes* nearly free, (surely not "adnate"), 1-1.7 cm. long, scissile, colonial buff to olive-ocher becoming citron green to lime green when exposed, with bluish tinges when bruised, drying dark olive buff to buffy brown; *mouths* circular (not "angular") when fresh, brick red

when looking into tubes, but morocco red when looking across them. The tramal tissues are of hyaline, septate hyphae which are  $5-6 \mu$  in diam., with occasional filaments which appear like lactiferous ducts. The tubes easily split from the pileus context. *Bardia* hyaline, 4-spored, broadly clavate to pyriform,  $24-28 \times 10-$

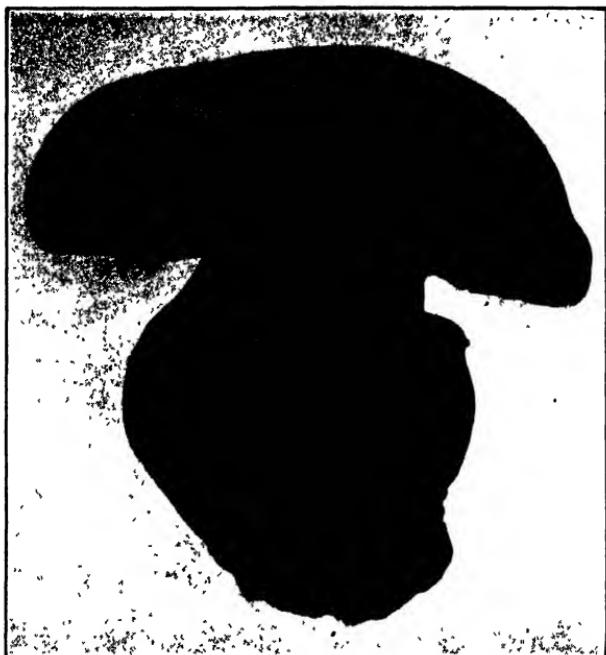


FIG. 3. *Boletus Eastwoodiae*.

$12.4 \mu$ ; *sterigmata* hyaline, short,  $3-4 \mu$  long, slender; *spores*  $10-14 \times 4-5.5 \mu$ , fusiform to narrowly ellipsoid, golden-yellow; *Cystidia* awl-shaped, rather sharp, granular, hyaline,  $17-22 \mu$  long; *stipe* 9-10 cm. long, 7-9 cm. broad in the middle, bulbose, solid, surface having yellowish under cast but mostly with pink tints (from orange-pink to light Corinthian red), flesh color with reticulations above.

The colored illustration of *Boletus purpureus* Fries (Atlas Pl. XII, Suppl. to Vol. 42, Bull. Soc. Myc. Fr. 1926) is very good for this collection except in the color of the pileus and flesh and reticulations on the stipe. It seems to have its closest relationships with *B. purpureus* Fries and *B. Queletii* Schulzer var. *rubicundus* R. Maire.

**24. *Boletus olivaceobrunneus* Zeller & Bailey sp. nov.**

Pileo 9-14 cm. lato, hemisphaeroideo vel convexo-expanso "olive-brown," centro leviter obscurioribus, vulnerata ad "fuscous-black" tarde mutante, aequo, sicco, breviter tomentuloso velutino spectans, leviter fissurato siccans; margine integro; carne usque 2.5 cm. crassa, firma vel spongiosa sordido-alba, cinerescens, sapore et odore miti foetide siccans; tubulis adnatis sinuatis interdum liberis fractans, primis subplanis avellaneis, demum depressis "snuff-brown" vel "bister," ore minute, 12 pro cm. circa, subrotundo, primo farcto, "buffy brown" vel "olive-brown"; stipite subcylindrico deorsum incrassato, 12-15 cm. longo, superne 2-3 cm. crasso, inferne 3-4 cm. crasso, superficie pileo pallidioribus longitrorsum costatuloso, superne reticulato, costae pileo concolore, solido, intus fibrilloso-carneosos sordido-albido, vulnerato ad brunneo-nigricans; sporis fusiformibus leibus, brunneo-ferrugineis,  $14-15 \times 5-6 \mu$ , guttulatis.

Ad terram in silvis Coniferarum prope Siletz (*F. D. Bailey*) et Newport, Lincoln County, Oregon, Amer. bor. October et November.

*Pileus* 9-14 cm. broad, hemisphaeroid to convex expanded, olive-brown (R) somewhat darker at the disk, fuscous-black where bruised, even, dry, minutely tomentulose giving a plushy appearance, cracking slightly on drying; *margin* entire; *flesh* up to 2.5 cm. thick, firm to spongy, sordid white becoming drab on exposure; *taste* and *odor* mild, drying fetid; *tubes* adnate, sinuate, sometimes breaking free, at length depressed, nearly plane, avellaneous when fresh, changing snuff-brown to bister, *mouths* small, averaging 12 per cm., subcircular, stuffed when young, buffy brown to olive-brown; *stem* subcylindrical, tapering upward, 12-15 cm. long, 2-3 cm. diam. above to 3-4 cm. below, solid, fibrillose-fleshy, sordid whitish changing brownish black within, surface slightly lighter than pileus, longitudinally ribbed, ribs concolorous with pileus, anastomosing, reticulate above; *spores* fusiform, smooth, brownish-ferruginous,  $14-15 \times 5-6 \mu$ , large guttulae.

Under *Picea sitchensis*, Siletz, October, *F. D. Bailey* (type); Newport, November, *Professor and Mrs. H. P. Barss*.

**25. *BOLETUS PINICOLA* (Vitt.) Rea.**

Under conifers, Parmelia Creek, Santiam National Forest, Linn County, May.

This is the first report of the species from Oregon, if not from North America. It answers the description perfectly, with the possible exception of the marginal white line which in ours shows merely as a semblance of a line in two or three short arcs.

**26. BOLETUS RETIPES Berk. & Curt.**

Under conifers near the Three Sisters in the Cascade Range, August. Collected by *W. E. Lawrence*.

The Oregon material answers the description in every way, except that the surface is cracked into truncated polygonal areolae, as described for *B. frustulosus* Peck. This is not the first time this character has been described for *B. retipes*, however. This is perhaps the first time this plant has been reported west of Wisconsin.

**27. FOMES ANNOSUS (Fries) Cooke.**

On down logs of *Acer macrophyllum*, Alsea Mountain, Benton County. Infrequent but this record of the species on a hard wood is of interest. December.

**28. POLYPORUS LAPPONICUS Romell.**

On dead wood of *Pinus contorta*, on the slopes of Mt. Hood in Hood River County. August. .

This plant has pronounced rosy tones so that one may at first feel that it can be referred to *P. fragilis* but it has much larger spores and differs in texture. I am taking the liberty to report this fungus under the name *P. lapponicus* Romell because of the following note from Dr. L. O. Overholts:

"Your No. 7546 would seem to be too near *P. ursinus* Lloyd (Synopsis Apus Polyporus, p. 319) to allow of separation. The species is common enough in the Rockies from Idaho to Colorado, but this is the first collection from west of the mountains. It also occurs in New England, New York, and Michigan, but apparently is a boreal species, going up to timber line in the Rockies. Some other name than *P. ursinus* will have to be applied to it, as it is not *P. ursinus* Link, from Brazil. *P. lapponicus* Romell is a synonym and probably the proper name for it."

**29. POLYPORUS OVINUS (Schaeff.) Fries.**

The following description is from fresh specimens:

*Pileus* circular to somewhat irregular, convex to plane or somewhat depressed at center, 5-9 cm. broad, 1-1.5 cm. thick at the

center, very thin at margins; *surface* even, tomentose at the center to squamulose appressed scaly, cream-buff to ochraceous-buff with dark reddish brown scales; *margin* very thin, even, somewhat undulating; *flesh* spongy, creamy white; *tubes* decurrent, 2-4 to the mm., angular, 1-2 mm. long, edges thin, fimbriate, straw yellow to empire yellow when fresh, cream color to wax yellow when drying, becoming deep olive buff to olive lake and deep Colonial buff when dry; *stipe* central sometimes excentric, equal, 3-8 cm. long, 0.7-2.0 cm. thick, usually a little lighter than the pores, sometimes with pinkish tints, staining brownish where bruised, solid with white flesh, smooth; *spores* smooth, hyaline, one large vacuole, broadly ellipsoid to subglobose,  $4-4.5 \times 3.5-4 \mu$ ; *odor* pleasant; *taste* mild.

In coniferous woods. Newport, October 18, 1931, *H. P. Barss.* Gregarious but rare.

This collection was identified by Dr. L. O. Overholts and consisted of 9 specimens. It is evidently a rare species, but widely distributed having previously been reported from Alabama, Colorado, and New York; and now from just above sea level in Oregon.

### 30. LEPIOTA CLYPEOLARIOIDES Rea.

In coniferous forest with some mixed deciduous trees, Lane County, October 24.

When the writer collected this beautiful species the solitary specimen was outstandingly distinct and easily identified. There was, however, some question concerning the exact color of the pileus of the European form but since then the determination has been confirmed by the Honorable Carleton Rea. The specimen conforms to Rea's description in every respect, and perhaps this constitutes the first report of the species from America.

### 31. *Armillaria badicephala* sp. nov.

Pileo 4-6 cm. lato, e convexo vel applanato, leviter umbonato; superficie sicca, parte centrali squamulosa badia vel "Sanford's brown," marginem versus pallide ochraceo-salmonia, radiatim squamis fibrillosis disco concolori; carne alba vel cremea, vulnerata ad "buff-pink" vel "onion-skin pink" tarde mutante, marginem sat tenui, centro crasso; sapore et odore leviter farinaceo; lamellis 6-7 mm. crassis, ex subaequalis semiellipticis vel semicirculis, albis ad "pinkish buff" tarde mutante, adnatis, remotiusculis, acie integris; stipite 5-7 cm. longo 8-12 mm. crasso, supra fibrillis arach-

noideis "pinkish buff," infra fibrillis striatis rufo-brunneis, cavo, intus albo vel "onion-skin pink; annulo arachnideo, evanido; sporis late oblongis vel subglobosis, levibus, hyalinis,  $5.5-7 \times 5-6 \mu$ .

Ad terram sub arboribus humilibus Coniferarum, prope Newport, Oregon, Amer. bor. Mensis Novembris.

*Pileus* 4-6 cm. broad, convex to plane, slightly umbonate; *surface* dry, squamulose on disk to fibrillose scaly toward margin, bay to Sanford's brown on the disk with scales toward margin concolorous on a background of light ochraceous-salmon; *flesh* rather thin at margin, thick on disk, white to creamy, slowly changing to buff-pink or onion-skin pink when exposed; *gills* 6-7 mm. broad, subequal or semi-elliptical to semi-circular in outline, white, changing to pinkish buff, adnate (sometimes sinuately-adnate) not crowded; *edges* even; *stem* 5-7 cm. long, 8-12 mm. in diam., striately-fibrillose scaly below the arachnoid, fugaceous; *ring*, arachnoid scaly above, scales reddish brown below, pinkish buff above, hollow, white to onion-skin pink within; *spores* short oblong to subglobose,  $5.5-7 \times 5-6 \mu$ , hyaline; odor and taste slightly farinaceous.

Under low scrubby spruce trees, Newport. November. Collected by Professor and Mrs. H. P. Barss.

For those to whom the genus *Armillaria* is untenable this species comes closer to the *Tricholoma* concept than the other white-spored genera. It has several characters in common with *Tricholoma subpessundatum* Murrill but differs in surface of stem and cap, and the presence of an annulus.

### 32. ELASMOMYCES MATTIROLIANUS Cavara.

Under conifers, Yaquina John Point, South of Waldport, Lincoln County, May.

There was but one fructification found. This came just even with the surface of the forest floor duff, in which it was immersed. The duff here had been tramped down and was rather firm. The fungus showed as a white spot about a centimeter in diameter but when dug proved to be about 2 cm. in diam., and was creamy white. The stipe and sterile tissues are vesiculose-pseudoparenchymatous; *basidia* 2-4-spored; spores subglobose, echinulate, 1-vacuolate, dilute straw yellow,  $7-11 \mu$ .

This is perhaps the first report of this species in America.

**33. PODAXIS PISTILLARIS (Pers.) Fries.**

In sandy irrigated strawberry patch west of Bend on the McKenzie Pass highway, Deschutes County. June.

This plant was found in this very hot, semi-arid district. There was only one specimen. The strawberries had been recently cultivated and this fruiting body was next to a strawberry plant where the cultivator did not disturb it. This extends the northern limit of distribution for this genus in western United States.

**34. MYCENASTRUM CORIUM (Guers.) Desv.**

In volcanic ash soil, among sage brush, near Redmond, Deschutes County. June. Frequent.

**35. CALVATIA SCULPTA (Harkn.) Lloyd.**

In open woods under conifers at high elevations in Crater National Park, Mt. Hood, Mt. Jefferson, and the McKenzie Pass in the Cascade Mountains. Early summer.

This interesting puff ball has previously been reported from California. It might easily be put in another genus because of its striking peridial characters. It is similar in these characters to *Areolaria strobilina* (Kalchbr.) Forq. but differs in having smooth spores and no definite stipe.

**36. LEUCOGASTER FOVEOLATUS (Harkn.) Zeller & Dodge.**

In rocky but peaty soil. Breitenbush Lake, Jefferson County. August. First report from Oregon.

**37. ALPOVA CINNAMOMEUS Dodge.**

Among humus or moss in the forests of the Olympic Mountains, Washington, Corvallis, Benton County and Mt. Hood, Oregon, and Trinidad, Humboldt County, California. October.

It is of extreme interest that so shortly after Dr. Dodge described *Alpova*<sup>4</sup> from Michigan, we find that collectors from the University of Michigan have also taken specimens of this genus

<sup>4</sup> Dodge, C. W. *Alpova*, a new genus of Rhizopogonaceae, with further notes on *Leucogaster* and *Arcangeliella*. Ann. Missouri Bot. Gard. 18: 457-464. illus. -1931.

in the States of Oregon and Washington. A note by Dr. C. H. Kauffman on collection No. 24 taken by L. E. Wehmeyer and Dr. Kauffman on Mt. Hood, Oregon, October 9, 1922, reads: "Among humus under log. Fruit body cream colored, then rufescent. Chambers at first filled with gelatinous substance; very gelatinous when mounted in water; becoming rufescent inside. Spores  $5 \times 2 \mu$ ."

A comparison of the western material with the syntype, No. Fp. 635, collected by A. H. Povah at Siskowet Outlet, Siskowet Bay, Isle Royle, Michigan, Sept. 4, 1930, leads to the conclusion that all should be incorporated in the one species, *A. cinnamomea*. To do this, however, some slight modifications of Dr. Dodge's description should doubtless be made. One of the Oregon specimens (No. 24) has a peridium  $750 \mu$  thick and the surface character is slightly different than described. In the Michigan material examined, as well as that from Oregon and Washington, often the peridium is much darker in the surface cells than farther inward, approaching a definite "rind" in appearance. The peridium is nevertheless always simple. The surface is very slightly pruinose.

The basidia are not always 8-spored. Five- to 11-spored basidia were definitely segregated both in the Michigan and western specimens. The spores are rarely  $7 \mu$  long, but mostly  $4-5.3 \times 2-2.5 \mu$ .

Since the above note was prepared a collection was brought to the writer from Trinidad, California. There were more than 20 sporophores in this collection and Mr. H. E. Parks says a hundred could be found in one small area. This material from Trinidad had so much the general appearance of *Melanogaster* that I looked through my own unidentified collections referred temporarily to this genus and found one of *Alpova* taken in an oak thicket, near Corvallis, June 30, 1926:

*Alpova* is an interesting genus and provides one more example of the occurrence of species both on the Pacific slope and the Great Lakes-Central Canada region.

Specimens examined:

Michigan: Isle Royale in Lake Superior, Siskowet Outlet at Siskowet Bay, Sept. 4, 1930, *A. H. Povah*, Fp. 635, Syntype (in Herb. Univ. Michigan and Zeller Herb.).

Washington: Chehalis Co., Quinault, Oct. 20, 1925, *C. A.*

*Brown* and *C. H. Kauffman*, No. 3 (in Herb. Univ. Michigan and Zeller Herb.).

Oregon: Benton County, Corvallis, June 30, 1926, *S. M. Zeller* 7047 (in Zeller Herb.), Oct. 9, 1922, *L. E. Wehmeyer* and *C. H. Kauffman*, two collections, No. 7 and 24 (in Herb. Univ. Michigan and Zeller Herb.).

California: Humboldt County, Trinidad, August, 1933, *Wendall K. Parks* (*H. E. Parks*, No. 4620, in Zeller Herb., 8191).

## IMPERFECTI

### 38. SPHAERONEMA PRUINOSUM Peck.

(Syn. *Glutinium macrosporum* Zeller, Jour. Agr. Res. 34: 489–496. 1927.)

On apple bark, Hood River and Marion Counties. September to March.

About two years after I described *Glutinium macrosporum*, Dr. W. C. Moore, Harpenden, England, asked for material to compare with a fungus he had obtained from apple in England. Dr. Moore turned the two over to Miss E. M. Wakefield who found the English and American fungi to be distinct, but she also found that the writer had overlooked an older name for the fungus, namely, *Sphaeronema pruinatum* Peck. In the meantime through the courtesy of Dr. H. D. House I have been privileged to examine a part of Peck's type from Garrison, N. Y., and a collection by Dr. House from Newcomb, N. Y. I agree with Dr. Wakefield that *G. macrosporum* is synonymous with *S. pruinatum* but I believe the fungus is not a good species of *Sphaeronema*. The pycnidia, as illustrated in my paper (citation above), are strictly cylindrical, within and without and are not rostrate. I have not, however, had opportunity to study the types of *Glutinium* and *Sphaeronema*.

### Neofuckelia Zeller & Goedding, gen. nov.

Stromate erumpenti, atro, discoideo vel pulvinato, subsessile vel stipitato; pycnidii stromate omnino immersis, unistratosis, ostiolatis; poro rotundo, leniter papillato; sporophoris simplicibus vel breviter ramosis; sporulis hyalinis, continuis, fusiformibus vel bacillaribus.

*Stroma* erumpent, black, discoid to pulvinate, subsessile or stalked, with several locules (pycnidia) wholly immersed in the stroma to the same plane; *pycnidia* ostiolate; *ostiole* slightly papillate; *conidiophores* simple or branched; *conidia* hyaline, continuous, fusiform or bacillar.

This genus perhaps falls most naturally into the Phomaceæ near *Dothiorella* because of the single stratum of pycnidia totally immersed in the black stroma. The latter, however, is usually stipitate in *Neofuckelia*, also a characteristic of the genus *Fuckelia*. In *Neofuckelia*, however, the pycnidia are ostiolate and in one stratum while in *Fuckelia* they are closed and merely locules without particular arrangement in a globose stroma.

### 39. *Neofuckelia pinicola* Zeller & Gooodding, comb. nov.

(Syn. *Fuckelia pinicola* Zeller & Gooodding, Phytopathology 20: 563. illus. 1930.)

*Stroma* erumpent, black, scattered to gregarious, pulvinulate, subsessile to short-stipitate, 0.8–1.2 mm. diam., with one stratum of 16–35 locules (pycnidia) wholly immersed; *stromatic tissue* of pseudoparenchyma surrounded by a carbonous rind of the same structure; *stipe* of same structure, short (or sometimes almost wanting); *pycnidia* ovoid to subglobose, somewhat crowded, walls prosenchymatous, lining the stromatic parenchyma, 60–150  $\mu$  broad, 200–260  $\mu$  deep, ostiolate; *ostiole* slightly papillate, 25–32  $\mu$  in diam.; *conidiophores* produced from entire inner surface of pycnidia, hair-like, simple or branched; *conidia* hyaline, continuous, fusiform, narrowly ellipsoid, or bacillar, 8–11  $\times$  1.7–3  $\mu$ .

Usually associated with *Atropellis pinicola* on cankered branches of living trees of *Pinus contorta*, *P. Lambertiana*, and *P. monticola*. Oregon, Washington, Idaho, and British Columbia.

One collection of stromata on stems of *Ribes Watsoniana* does not seem to differ from *N. pinicola*. This material was taken by G. D. Darker and L. N. Gooodding east of Mt. Hood, Hood River County, Oregon, July 11, 1929.

### 40. *DIPLODIA SYCINA* Mont. var. *SYCONOPHILA* Sacc.

On honey fig (*Ficus Carica* var.) causing a die-back of the small twigs. Newberg, Yamhill County. January. Infrequent.

This is the first time this disease has been reported from Oregon.

**41. BOTRYODIPLODIA CONGESTA (Lév.) Sacc.**

On bark of *Juglans californica*, Corvallis. April.

The stubs where this fungus was plentiful on the bark had been grafted to the Persian walnut but the scions failed. The fungus may have caused the death of the bark on the stock. Several cases were observed.

**42. Melanconium candidum (Peck) comb. nov.**

Syn. *Melanconium bicolor* var. *candidum* Peck.

*Acervuli* discoid to broadly conic, black, erumpent, 1–1.5 mm. in diameter; *stroma* central, truncate-columnar, 240–320  $\mu$  broad at top, 400–800  $\mu$  broad across the discoid base, creamy-white, of hyaline prosenchyma; *locule* circinate-conic about the ectostroma, surmounted by the periderm; *conidiophores* from all walls of the locule, simple to branched, short, hyaline to brownish; conidia ovate, fuscus, smooth, 8–11  $\times$  7  $\mu$ .

In bark of *Alnus rubra*, 20 miles south of Port Orford, Oregon, and Bainbridge Island, Washington. February to July.

This species was first described by Peck<sup>6</sup> as *Melanconium bicolor candidum*, but we believe it should be raised to specific rank for it is very distinct from *M. bicolor* Nees. The ectostroma is not so white as in the latter. It is creamy and the spores are 8–11  $\times$  7  $\mu$ , while in *M. bicolor* they are 14–17  $\times$  6–8  $\mu$ . This is distributed in *Fungi Columbiani*, 3334, as on *Alnus rubra* and the type in the N. Y. State Museum is on *A. rubra* but in Peck's description (l. c.) it is reported on "Bark of red mulberry, *Morus rubra* L."

**43. GLOMERULARIA CORNI Peck.**

Leaf-spot of *Cornus canadensis*, Sherwood Forest Camp, Mt. Hood Loop Highway, Hood River County, June. Collected by Roderick Sprague and F. D. Bailey.

Common in this type of mountainous location in the Northwest. To our knowledge not previously reported west of the Adirondack Mountains.

<sup>6</sup> New York State Mus. Bull. 150: 65. 1911.

**44. RAMULARIA DESTRUCTANS Zinnsm.**

Causing root-rot of *Panax quinquefolium*, Mayger, Columbia County. Infrequent. Late summer and fall.

**45. RAMULARIA RUBICUNDA Bres.**

On leaves of *Maianthemum bifolium* D. C. var. *kamtschaticum* (Gmel.) Jepson. Widely distributed in the Coast Range and westward. May to July. Very common.

This is a very severe leaf spot of this host. It has been observed from Curry County in Oregon to King County in Washington.

# SPHAERIA ZEAE (DIPLODIA ZEAE) AND CONFUSED SPECIES

C. L. SHEAR AND N. E. STEVENS

(WITH 2 TEXT FIGURES)

## SCHWEINITZ' FIRST USE OF THE NAME SPHAERIA ZEAE

Schweinitz (16) in 1822 described his first *Sphaeria Zeae*, as follows:

"234 [*Sphaeria*] *Zeae* Sz.

*S. minuta aggregata conferta conica tuberculosa sulcata atra, intus succo repleta, villo albicante circumdata.*

*Frequentissime provenit ad Zeae caulinum emortuorum nodos vere et eos inquinat. Atra quasi rubescens. Siccata quasi spinulosa redditur.*"

In a manuscript of Schweinitz preserved at the Philadelphia Academy of Science, entitled, "Cryptogamarum praestantiorum Index novissime inventarum December 1820" is the following description of the same fungus:

"X 204. *S. Zeidis* Nobis.

*S. minuta aggregata conferta et sparsa plerumque conica, tuberculosa, atterima sed quasi rubescens-sulcata, sicco-intus fusco fructifero repleta-villo albido evanescente circum elata. Sicca tactu quasi spinulosa. Frequentissime ad nodos caulinum Zeidis provenit.*"

This is evidently a slightly modified form of the description published later and quoted above; the form of the specific name having been changed in the published work.

In another manuscript of Schweinitz at the Philadelphia Academy, entitled, "Comentaria in Pyrenomycetum Friesii Species quae hic usque in American boreali obviae factae Sunt Ludovico Dav. de Schweinitz additis descripsionibus novarum 1828," is found the following record:

"*D. [othidea] Zeae* L.v.S. Syn. Car. *Sph. Zeae* n. 234 non *rara* Carol. (nec. *Perisp.*) circum nodo caulinum zeae (caude distinguenda *Cladospor.* herbar. saepe in taliter aut aliter quasi caespitulatum indurescens) omnino haec pertinet."

Later (1832) Schweinitz (17) published a slightly modified description of the above form as follows, citing the original description of his No. 234 quoted above.

"1866. 1. *D. [othidea] Zeae*, L.v.S., Syn. Car. no. 234, *Sphaeria*, frequens in Carolina nec in Pennsylvania obvia circum nodos caulis Zeae. Omnino hujus loci. Caule distinguenda a *Cladosporio* herbaceo indurato frequenter occurrrens iisdem locis."

All the above descriptions of Schweinitz apply clearly to *Gibberella Saubineti* (Mont.) Sacc. and an examination of his specimens confirms this conclusion.

The original packet in Schweinitz' Herbarium at Philadelphia labelled "Sph. Zeae LvS. Salem" is empty, only a bit of cornstalk pith remaining glued to the paper.

Schweinitz' autograph specimen in Hooker's Herbarium at Kew which Schweinitz sent him in 1824 has been examined by the writers. It shows both ascospores and conidia of *Gibberella Saubineti*. This identification was first made in 1859 by Curry (4) in his report of a study of the same specimen (FIG. 2) which bears his notes. Curtis also examined Schweinitz' specimen of this species and has a marginal note in his copy of Schweinitz' North American Fungi opposite No. 1866, as follows: "Sph. saubineti M." In Michener's Herbarium, Mycological Collections, Bureau of Plant Industry, there is also a part of Schweinitz' original collection of this No. 1866 labeled "*Dothidea Zeae Schw.*" This is also *Gibberella Saubineti*.

Schweinitz now having transferred his original *Sphaeria Zeae* to *Dothidea*, as indicated above, apparently considered the name eliminated from the genus *Sphaeria* and therefore free for application to another plant under the same genus.

#### SCHWEINITZ' SECOND USE OF THE NAME SPHAERIA ZEAE

In 1822 Schweinitz (16) also mentioned another *Sphaeria* on cornstalks, as follows:

"79. [S.] striaeformis  $\alpha$ ) frequens in Arundinaria macro-sperma;  $\beta$ ) in Juncis rario;  $\gamma$ ) in caulis Zeae rarissima."

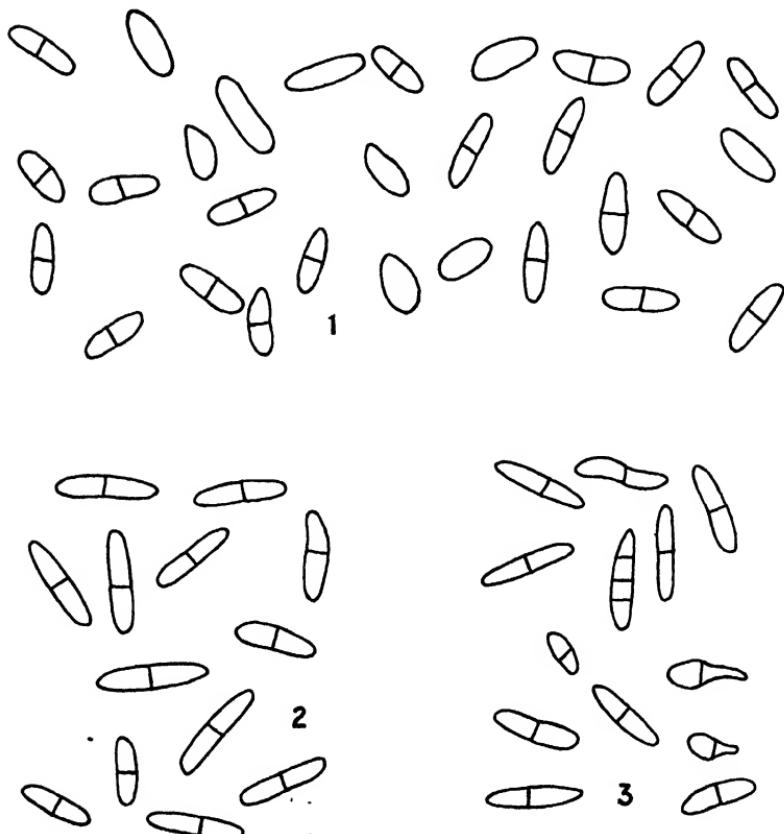


FIG. 1. 1, spores from one of Schweinitz' specimens of his second "Sphaeria Zeae" in the mounted collection at Philadelphia, apparently an aberrant form or a distinct species,  $\times 400$ ; 2, spores from Schweinitz' specimen of *Sphaeria Zeae* in Michener's herbarium in Washington regarded as the type,  $\times 400$ ; 3, spores from *Diplodia Zeae* (Schw.) Lév. on corn stalks from near Charlotte, North Carolina. 1934.  $\times 400$ .

Later in Schweinitz' manuscript, "Comm. Pyren." etc. 1828, already referred to, this is described as follows:

"273. S. Zeae LvS. Syn. Carol. n. 79 S. striaeformis, rara sed distinctissima in caulis Zeae—S. epidermide elevato fuscotincta omnino nisi ostiolis tecta-seriatum disposita-brevis sed subconfluen. utrinq. acuminat. Perithecijs in quoque caespitulo unis vel ternis subdistinctibus-ostiolis latis umbilicæ

saepe unico intus prim. albofarcis dene cavis.  
Nond. In Penyslv. observum."

In 1832 he (17) published a slightly modified form of the above description as a new species, his second *Sphaeria Zeae*, as follows:

"1451. 306. S. Zeae, L.v.S., Syn. Car. 79, rara sed bene distincta in caulibus Zeae, Salem et Bethl. S. omnino tecta, epidermide fusco tincta (ostiolis solis prominulis) satis elevata. Seriatim disposita, brevis, utrinque acuminata, subconfluens. Peritherciis binis vel ternis tantum in caespitulo, subdistantibus, primum albofarcis, demum evaeuatis. Ostiolis latis, umbilicatis, saepe unico."

The original packet of this number in Schweinitz' herbarium labelled "Sphaeria Zeae L.v.S. Salem" is empty. Schweinitz himself sent some of it to Hooker (18). Later, Curtis took a part and finally Michener (19) divided the remainder, mounting part of it and keeping a bit for his own herbarium.

The following specimens referred by Schweinitz to his second *S. Zeae* have been examined by the writers:

There is a specimen in the mounted collection of Schweinitz' fungi at the Philadelphia Academy of Sciences, Philadelphia, showing the remains of a gummed paper strip which the writers (18) have shown to be found only on his earlier specimens, which were probably collected at Salem, N. C. There is also another specimen from Schweinitz which is apparently a part of the above, in Berkeley's herbarium at Kew marked "S. (seriata) Zeae L.v.S." and another part of the same specimen in the Curtis herbarium at Harvard. All of the above specimens bear pycnidia of a fungus resembling *Diplodia Zeae* of present authors. So far as can be determined from these scanty specimens the structure of the pycnidium is identical with the common form. The spores, however, differ from any other specimen thus far examined from any source, in the presence of numerous spores which are much wider in relation to the length and somewhat wider absolutely than those in typical *D. Zeae*. These wider spores are either one or two-celled. Figure 1 shows the range in shape and size of spores from these specimens. In size they range from 14.5–26  $\mu$  in length and from 3–9  $\mu$  in width. Having been unable to find any other specimen of this form in numerous collections on cornstalks from

North Carolina and elsewhere, we are at present unable to say whether this is a different species from the common *Diplodia Zeae* or an aberrant form of it.

While no fungus having a spore variation of this type has yet been seen by the writers, certain variations are very common in the ordinary *Diplodia* on corn. Spores with 2 or even 3 septa are not uncommon. One celled spores are found in some specimens and as already noted one cell of a spore is frequently smaller than the other. Figure 1, *B* shows the range of spore form in a single mount of what we regard as typical *D. Zeae*.

In Hooker's Herbarium at Kew there is also an autograph specimen of Schweinitz' labelled "Sp. cristata on *Zea* 1." Below this label in another hand is "*Diplodia Zeae* Lév. (*Doth. Zeae* Schw. Syn. U.S.)" and "*Sph. Zeae* Schw. Syn. U.S." On this specimen there are two fungi. Below the node on the stalk is typical *Diplodia Zeae* of recent authors. Above the node is a *Leptosphaeria* sp., to which Schweinitz apparently intended to apply the name *cristata*.

In the Michener Herbarium there is a Schweinitz specimen labelled "*Sph. Zeae* Schw. in *Zeae* Sal.-Beth." This bears good pycnidia of typical *Diplodia Zeae* of recent authors and is the specimen which we believe should be accepted as the type of *D. Zeae* (Schw.) Lév. as it agrees with the present general conception of this species, generally one septate, rather variable in length, but shows few irregularities in shape, except for the fact that one cell of the spore is frequently less well developed than the other. In size they are  $24-29 \times 5-6 \mu$  (FIG. 1, *B*).

#### LATER STUDIES OF THESE FUNGI

Berkeley (2) in 1847 received specimens of a fungus on corn-stalks from Ohio which he described as follows:

"139 *Sphaeria* (Seratae) *Maydis*, n. sp.; maculis parvis subellipticis elevatis; peritheciis paucis; ostiolo unico conico, sporidiis oblongis curvulis uniseptatis.

On dead culms of *Zea Mays*. Cincinnati, Ohio. May 1, 1841. T.G. Lea, Esq.

Habit that of *Sphaeria Arundinaceae*. Spots minute, often purple-brown, punctiform, or subelliptic, rarely linear, con-

taining very few perithecia, with a single broad conical ostiolum. Sporidia oblong, slightly curved, uni-septate. Very different from *Sphaeria Zeae*, Schwein. ! as appears from an authentic specimen in Sir W. Hooker's Herbarium."

The *Sphaeria Zeae* Schw. referred to above by Berkeley was the first of Schweinitz' plants of that name which has been shown to be *Gibberella*. He apparently at that time had not studied Schweinitz' second *Sphaeria Zeae*. Berkeley's type which we have examined, is typical *Diplodia Zeae* of present authors.

In 1848 Léveillé (11) described Schweinitz' second *Sphaeria Zeae* as *Diplodia Zeae* as follows:

"*Diplodia Zeae*, n. sp. Conceptaculis gregariis innatis ovatis intus extusque nigris epidermide nigra tectis, ostiolis erumpen-acutis; sporis elliptico-elongatis rectis vel curvatis subopacis.-Hab. Tete de Buch prope Burdigalam ad culmos *Zeae Maydis*.

*Sphaeria Zeae* Schweinz. Syn. Fung. North Amer., p. 207—  
*Sphaeria dolosa* Pers. (herb. Lugd. Batav.)

Desc. conceptacles assez rapproches, globuleux au ovales, nichés dans l'épaisseur du chaume, et recouverts chacun par une portion d'épiderme noir, Ostioles aigus, proéminents à travers la rupture de l'épiderme. Substance intérieure de couleur noire, composée de spores allongées, obtuses à une extrémité, aiguës à l'autram droites au courbées avec une colison médiane.

*Obs.* La ressemblance de ce *Diplodia* avec une sphérie cauli cole est frappant: mais quand on la soumet au microscope, l'illusion disparaît: on voit alors qu'elle n'a de thèques, et que ses spores sont supportées par un clinode, dont les basides sont à peine visibles."

The above description leaves no doubt that Léveillé had the fungus to which this name is now applied in this country.

Currey (4) in working over the *Sphaerias* of Hooker's Herbarium at Kew examined Schweinitz' autograph specimen of his first *Sphaeria Zeae* (FIG. 2) and later (5) identified it as "nothing more than *Sphaeria pulicaris* Fr." (*Gibberella Saubineti*). He also examined Berkeley's type of *Sphaeria* [*Diplodia*] *Maydis* collected by Lea in Ohio and pointed out that it was entirely different from the *Sph. [Gibberella] Zeae* Schw.

Hazslinsky (8) in 1873 described and illustrated *Diplodia Zeae*

from specimens collected on cornstalks in Hungary and called it *Hendersonia Zeae* Currey.

Ellis and Everhart (7) in 1892, after examining a specimen of

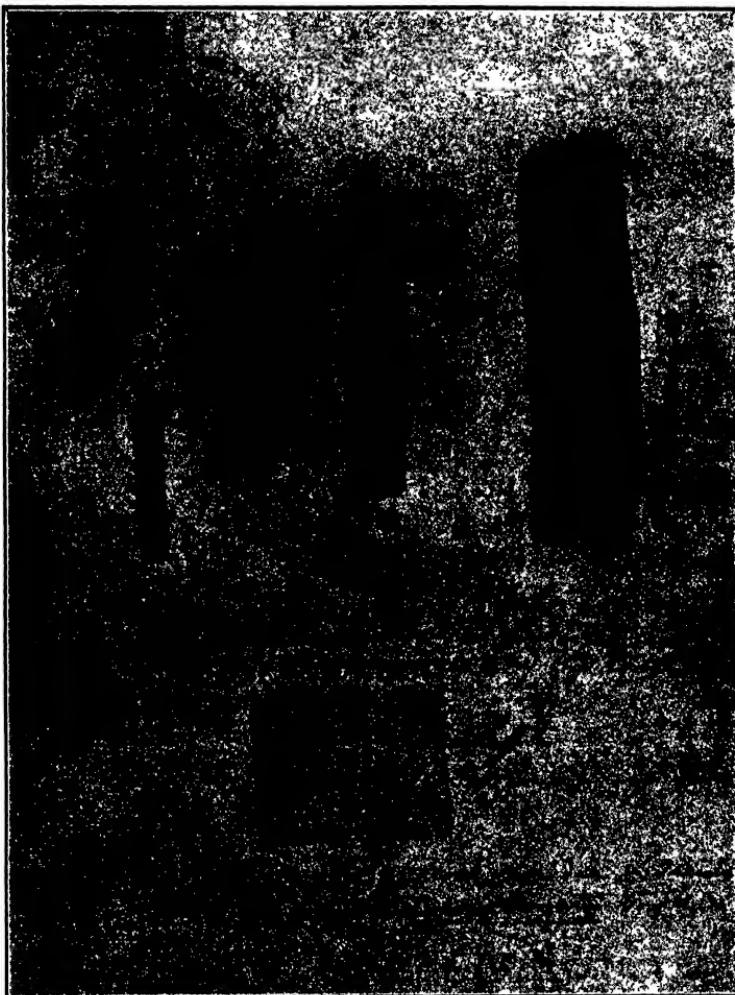


FIG. 2. Schweinitz's autograph specimen of his first *Sphaeria Zeae* with Currey's notes.

Schweinitz No. 1451, his second *Sph. Zeae*, at the Philadelphia Academy say it "is the same as *Diplodia Zeae* Lév. in Ell. N.A. Fun. Exs. No. 31." This has been verified by an examination of

this number in the Mycological Collections of the Bureau of Plant Industry.

Under the name *Diplodia Zeae* (Schw.) specimens were distributed by Ravenel as No. 74 of his *Fungi Caroliniani* Exs. in 1852, and again in his *Fungi Americani* Exs. No. 393 in 1879. Ellis also distributed it in his *N.A. Fun. Exs.* No. 31 in 1878 as *Diplodia Zeae* Lév. and also in Ellis and Everhart *Fun. Col. Exs.* No. 73, 1893, under the same name. The specimens of the above numbers in the Mycological Collections, Bureau of Plant Industry, have been examined and are all typical *Diplodia Zeae* (Schw.) Lév.

In 1888 Bennett (1) listed this fungus as "Dothiora zeae Sw." according to Burrill and Barrett (3).

In January 1909 Heald, Wilcox and Pool (9) described and illustrated this fungus and discussed its synonymy, deciding that the proper name was *Diplodia Zeae* (Schw.) Lév. There is no evidence, however, that they examined any type or authentic specimens of Schweinitz.

Burrill and Barrett (3) later in the same year discussed the synonymy, but also without examination of any type or authentic material. They assumed that all Schweinitz' specimens under the name *Sphaeria Zeae* referred to the same plant and they used the same name as Heald, Wilcox and Pool, *Diplodia Zeae* (Schw.) Lév.

In both the papers just mentioned, there are adequate illustrations of the pycnidia and pycnospores, those of the earlier paper being particularly good. The spore measurements as given by Heald, Wilcox and Pool (9) are  $24-33 \times 5 \mu$ . Burrill and Barrett (3) noted a variation in the length of the pycnospores depending somewhat upon the culture medium used, giving the length as 22.1-28.5 and the width rather constantly at 5 and 5.2. These two papers were the first in which the pathological importance of this fungus was considered.

Saccardo (15), 1913, follows Heald, Wilcox and Pool, and adopts the name *Diplodia Zeae* (Schw.) Lév. for this fungus. He (14) had in 1884 used the synonym, *Diplodia Maydis* (Berk.) Sacc. for this species.

Spegazzini (20) in 1910 described "*Diplodia* (?) *maydicola*" as a new species. According to the original description this

agrees with *D. Zeae* (Schw.) Lév. Spore measurements are given as  $24-26 \times 5-7 \mu$ , and though the writers have not seen Spegazzini's specimen they do not doubt its identity. Von Höhnel (10) in 1918 also expresses the same opinion in regard to Spegazzini's species.<sup>1</sup>

Woronichin (22) in 1922 proposed the new generic name, *Hendersoniopsis*, for this species. Later he found that name had been used by von Höhnel in 1918, and must be changed. In 1925 he (23) found the fungus on *Zea Mays* in Russia and changed the name to *Phaeostagonosporopsis*. None of his material has been seen but from his statement that it agrees with the descriptions and illustrations of Burrill and Barrett, and Heald, Wilcox and Pool, it is clear that his fungus is the same as ours.

Petrak and Sydow in Petrak (12) named the fungus *Macro-diplodia Zeae* (Schw.) P. & S. In 1926 the same authors (13) described the species in detail after having examined type and authentic specimens from Kew. The specimen labelled "S. (seriata) zeae" which was part of the early collection of Schweinitz' second *Sphaeria Zeae*, they regarded as a small spored form of *Diplodia Zeae* (Schw.) Lév. Their examination of the specimen labelled "Sph. cristata in Zeae" to which they refer as "Sph. aristata" gave the same result as we have indicated above.

While the fungus which is here regarded as *Diplodia Zeae* (Schw.) Lév. is not a typical *Diplodia* in the sense in which the present writers are using the name, that is, for the pycnidial forms of *Physalospora*, until its complete life history is known it does not seem desirable to transfer it to another genus as Petrak and Sydow, and Woronichin have done. The synonymy so far as known at present is as follows:

#### SYNONYMY

- 1822 *Sphaeria striaeformis* var.  $\gamma$  Schw.
- 1832 *Sphaeria Zeae* Schw.
- 1847 *Sphaeria Maydis* Berk.

<sup>1</sup> Since this was written Stevens, N. E. and Hoppe, P. E. (Plant Dis. Rep. 19: 70, 1935) have obtained numerous cultures of *D. Zeae* from corn grown in Argentina, Uruguay and Mexico. The fungus probably occurs wherever *Zea Mays* is grown.

- 1848 *Diplodia Zeae* Lév.  
1859 *Sphaeria (Hendersonia) Zeae* (Schw.) Curr.  
1873 *Hendersonia Zeae* (Curr.) Hazsl.  
1884 *Diplodia Maydis* (Berk.) Sacc.  
1888 *Dothiora Zeae* (Schw.) Bennett.  
1923 *Macrodiplodia Zeae* (Schw.) P. & S.  
1925 *Phaeostagonosporopsis Zeae* (Schw.) Woronich.

*Diplodia macrospora* Earle (6) regarded by Petrak and Sydow as a variety of this species, while obviously closely related, has spores twice as long and seems to the writers deserving specific rank, as we have never found any intergrading forms.

From the facts above presented it is obvious that if the present International Code were followed literally, it would be necessary to apply the specific name *Zeae* Schw. to the fungus now generally known as *Gibberella Saubineti*, and the name *Maydis* Berk. to the fungus now nearly everywhere known as *Diplodia Zeae* (Schw.) Lév. The absurdity of such a procedure seems self-evident. Such changes could serve no useful purpose either scientific or otherwise and would only add to the confusion already too prevalent in mycological nomenclature. Since uniformity and stability in the use and application of names is the primary aim of nomenclature, *Diplodia Zeae* (Schw.) Lév. which has been the general and practically uninterrupted usage for over 50 years should continue to be the name applied to the fungus under consideration at least until its perfect stage, if any, is discovered.

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# TETRACLADIUM MARCHALIANUM AND ITS RELATION TO ASTEROTHRIX, PHY- CASTRUM, AND CERASTERIAS

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(WITH 5 TEXT FIGURES)

## INTRODUCTION

In connection with studies to determine the susceptibility of species of *Isoetes* to *Cladophytrium replicatum* Karling a large, unusual aquatic hyphomycete was isolated from dead and decaying leaves of *I. lacustris* growing in the greenhouse tanks at Columbia. This fungus proved to be so unique and striking as to the size and shape of its conidia as well as their method of development that it was at first believed to represent a new genus and species of Fungi Imperfecti. Subsequent study of its development and morphology and a survey of the literature have shown, however, that it is undoubtedly identical with *Tetracladium Marchalianum*, which was discovered and established by De Wildeman in 1893. This saprophytic and monotypic genus was founded on material discovered among algae and the debris of higher plants in a pond in the Botanical Garden of Brussels, and since that time, because of its confusion with several genera of algae, has been the object of extensive discussion and controversy. De Wildeman noted at once the similarity of the conidia to the algae *Phycastrum longispinum* described by Perty in 1852 and *Cerasterias raphidioides* by Reinsch in 1867 and 1888, and expressed considerable doubt as to whether the organism in question was an alga or fungus. He submitted a sketch of this organism to Saccardo, who expressed the opinion that it was undoubtedly a fungus and closely related if not identical with his *Titaea callispora*. De Wildeman described it again in 1894 from stems of *Hippuris vulgaris*, classed it in the Phragmosporeae division of the family Mucedinaceae, and reported its further occurrence in France and

Switzerland. He again pointed out the striking resemblance of the conidia to the above mentioned algae, and particularly *Astrothrix microscopica* Kütz (1845-1849). In the following year Chodat, commenting on the forms which he had observed for several years in Geneva, maintained that *Tetracladium* and *Cerasterias* are identical and that the latter generic name should be excluded from the algae, on the grounds presumably that the organism to which it applied was not an alga but a fungus. In reply (1895) to Chodat's contention De Wildeman agreed that *Tetracladium Marchalianum* is synonymous with *Cerasterias raphidioides* var. *incrassatum* and *inaequale* as described by Reinsch in 1888 but quite different from the forms reported in 1867, and maintained that the genus *Cerasterias* should be retained in the algae for these earlier species.

Saccardo recognized De Wildeman's genus *Tetracladium* in 1899 and transferred it from the Phragmosporeae to the Staurosporeae division of the Mucedinaceae, next to *Titaea*, but in 1900 and 1907 Lindau referred it to the Phragmosporeae again, a grouping which has very recently been followed by Migula (1934). Clements and Shear (1930), on the other hand, place it in the Staurosporeae group of the family Moniliaceac.

In the meantime Printz (1914) revived again the questions of identity and synonymy of this organism. In material collected by Wille in Norway he found thalli of *Cerasterias raphidioides* which, from his drawing, appear identical with those of Reinsch of 1888, and he is convinced that this species is an alga, should be transferred to the genus *Astrothrix* Kütz, and is very similar to and possibly identical with *A. longispinum* or *A. tripus*. If the latter view is correct we would thus have a most confusing synonymy for *Tetracladium Marchalianum* De Wild., which would include indisputable algal species. In reply to Printz's claims De Wildeman (1920) called attention to this confusion and reasserted his previous contentions that *Cerasterias raphidioides* var. *incrassatum* and *inaequale* or *Tetracladium Marchalianum* is without question a fungus. In 1925, however, this species was reported again from Switzerland by Huber-Pestalozzia, who accepted Printz's synonymy and described it as *Astrothrix (Cerasterias) raphidioides* (Reinsch) Printz. Huber-Pestalozzia apparently never grew this

organism in culture, and his entire study is devoted to the variations and growth of the conidia or spores which he regards as individual thalli or plants. Although he figured and described these structures in considerable detail and believed he saw new thalli originating at their apices, he was still uncertain as to the systematic position and fungous nature of this species and expressed the opinion that the genus *Astrothrix* might represent a group of primitive fungus-like organisms.



FIG. 1. Variations in the size and shape of conidia of *Tetracladium Marchalianum* De Wild.

Since that time *T. Marchalianum* has been reported from Canada by Lowe (1927) in a brief note entitled "Cerasterias, the Child of Sorrow of the Algologists." Spectrum analysis and culture work by Lowe proved conclusively the absence of chlorophyll in the spores and that the latter are true conidia which germinate readily on ordinary fungus media and produce well developed mycelial colonies within 48 hours. Unfortunately, Lowe gives no figures of the form he described. In the same year Printz (1927), apparently realizing the fungous nature of this species, excluded the entire genus *Cerasterias* from the algae and admitted the

synonymy of *C. raphidioides* and *T. Marchalianum*. However, he makes no distinction between *C. raphidioides* forms *tridens*, *tetradens*, *octodens*, and *obtusa* Reinsch of 1867 and *C. raphidioides* var. *inaequale* and *incrassatum* Reinsch of 1888, and excludes both of them as being fungi. In 1928, however, Collins listed the latter as an alga again and copied one of Reinsch's (1888) figures to illustrate the species as it occurs in North America. Except for Smith's (1933) statement that *C. raphidioides* var. *inaequale* and *incrassatum* is a fungus, subsequent reports of this species are lacking, as far as I am aware, and up to the present time it has been described from Germany, France, Belgium, Switzerland, Africa, Norway, Dutch Guiana, Canada, and the United States.

In view of the long controversy over the identity of *T. Marchalianum* and its confusion with the algal genera *Phycastrum*, *Polyedrium*, *Cerasterias*, and *Astrothrix*, the author feels that a brief description of the structure, development, and cultural characteristics of our form is highly worth while and necessary to demonstrate conclusively the fungous nature of this species and to clarify its position relative to the algae. Cytological studies on the structure of the mycelium and the development of the conidia are in progress and will be reported later.

#### STRUCTURE AND DEVELOPMENT OF THE CONIDIA, CONIDIOPHORES, AND MYCELIUM

As has been shown by De Wildeman, the conidia of *Tetracladium Marchalianum* are strikingly unique in shape and structure. They are hyaline, multicellular, flat, somewhat triangular in shape, and brush-like in appearance, with the basal or attachment cell serving as the handle. As to septation they are essentially phragmosporous, no dictyosporous conidia having so far been observed. Text figure 1 shows the range of variation in size and shape of conidia from corn meal agar cultures. The same range of difference has also been found in nature on decaying *Isoetes* leaves. As shown in this figure, the individual spores consist of from 8 to 18 cells extending out in a more or less flat plane at the apex of a central axis. Text figures 1A, 1B, 1E, and 1F show comparatively small conidia, while in the remaining figures they are more complex.

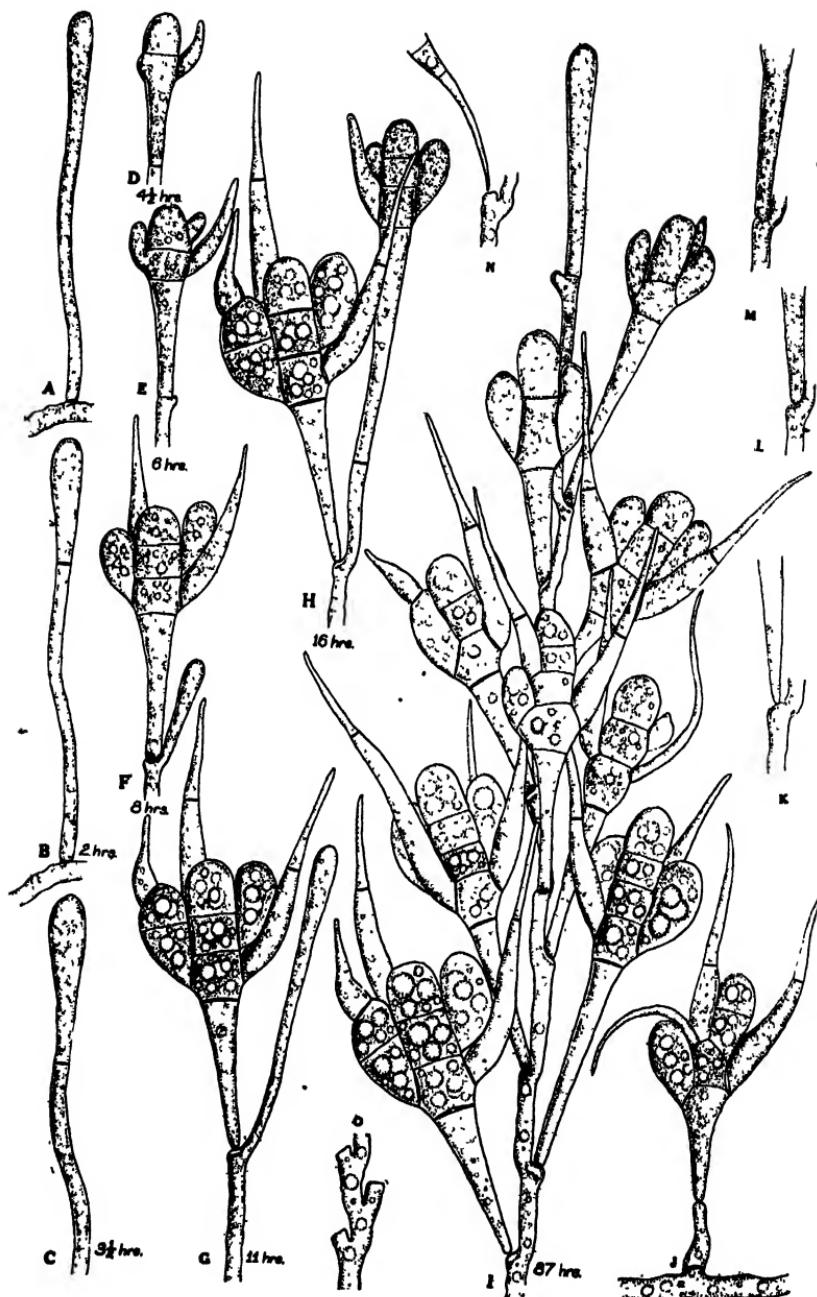


FIG. 2. Successive stages in the development of a conidiophore and conidia.

A conspicuous feature of all conidia is the presence of from one to four finely drawn out or needle-like cells, which extend considerably beyond the main body of the spore. The basal cell by which the conidia are attached to the conidiophores may also become greatly extended and pointed at maturity, so as to be indistinguishable from the more apical spines. All these needle-like extensions are very soft and pliable in spores which have just been formed, and may readily be bent and displaced by dissection needles. As the conidia grow old and become more or less dry, the spines may readily break off (FIG. 1K). In the majority of conidia one of the spines is usually more fine and stylus-like, and closely resembles the stylospores of *Phomopsis*. They generally break off from the conidia at maturity likewise, and in agar cultures may be found quite numerous in the vicinity of the spores. Figure 1E shows such a stylus-like spine attached to a conidium, while in 1I and 1J they are shown detached.

The other cells of the conidia are more or less globular in shape. At maturity they are generally filled with large and small refractive globules which may become so numerous as almost to obscure the cross septa in living material. Quite frequently they fill the cells completely and by mutual contact and pressure become more or less hexagonal in shape. Such globules may also be present in the bases of the spine cells, but it is not uncommon to find the latter completely hyaline. When viewed from the side, as in figure 1F, the conidia appear somewhat flattened, showing that the cells do not extend out in all planes. Due to their unusual shape and structure, it is of course difficult to give representative measurements of the spore. The large conidium shown in figure 1H measured 75  $\mu$  from tip to tip, and 22  $\mu$  at its broadest diameter; while those illustrated in figure 1A and 1B were 28  $\mu$  and 32  $\mu$  in length. De Wildeman found variations in length of from 10 to 108  $\mu$ .

The conidiophores are comparatively short and usually arise at right angles to the mycelium as somewhat club-shaped branches. The majority are single and unbranched, but numerous branched ones have been found both in agar cultures and on *Isoetes* leaves in nature. A series of successive stages of development of a conidiophore and conidia at different time intervals is shown in

figure 2 of material in a hanging drop chamber. The apical end of the conidiophore begins to enlarge (FIG. 2A-2C) and forms a cross wall which thus delimits the initial of the primary spore. Very shortly thereafter this initial may undergo further transverse septation (FIG. 2D, 2E) and develop buds or short branches which in turn are delimited by septa. At this stage the cytoplasm is quite dense and finely granular, and numerous small refractive globules begin to appear. These increase in size and number as the conidium develops, until at maturity, as noted before, they may be so large and numerous as to fill the cells entirely. By development of additional buds or branches and their growth and division in one plane (FIG. 2E-2G), the mature conidium is

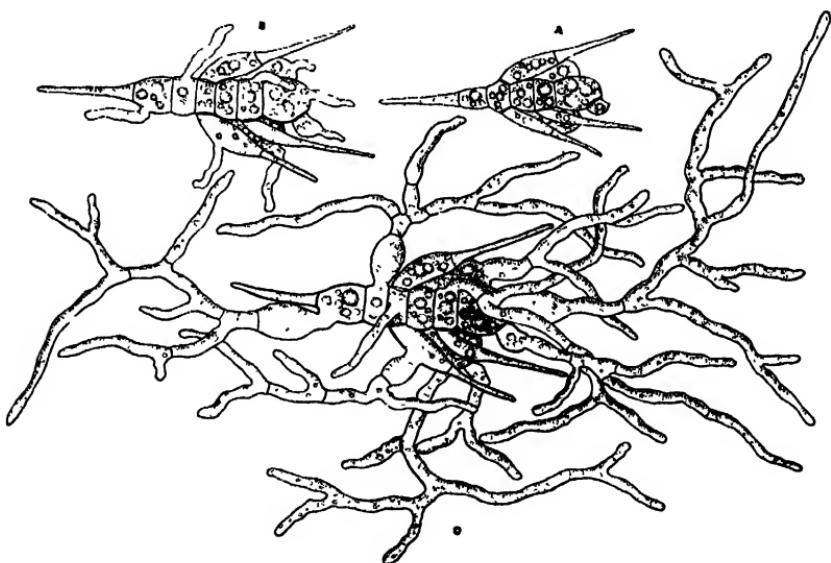


FIG. 3. Successive stages in the germination of a conidium.

formed in from 7 to 12 hours. Quite often the spine initials appear first (FIG. 2D) as more slender buds, but there seems to be no regular sequence in their appearance with respect to the other cells. At maturity they may or may not become septate and multicellular. It is obvious from this description that the multicellular condition of the conidia does not result from repeated divisions of a mother cell in a meristogenous manner, but fundamentally by the development, delimitation, and growth of buds or short branches.

In the meantime the conidiophore beneath has continued growth and formed the initial of the second conidium (FIG. 2E-2G). The latter is very shortly delimited by a septum and develops in the same manner as the primary spore (FIG. 2H); while the conidiophore continues on to form additional conidia in acropetal succession (FIG. 2I). In agar cultures, as far as my observations go, it is rare to find more than five and six conidia on a single conidiophore. Two, three, and four are the usual numbers. Oftentimes they may be borne singly on short conidiophores, as is shown in figure 2J. The conidiophore illustrated in figure 2I is exceptional, according to my observations, and doubtless represents a maximum of complexity and development. In this figure are shown 9 conidia with the 10th in an incipient stage at the apex. As a result of this acropetal method of conidial development, the conidiophore may be somewhat irregular or slightly zigzag in outline with marked shoulders where the successive spores were borne. Quite frequently the shoulders may be so prominent (FIG. 20) as to look like short branches.

Particularly noteworthy are the changes which take place in the shape of the basal or attachment cell as the conidia mature. While attached to the conidiophore the basal end is rather blunt (FIG. 2M), but as maturation continues it becomes finely drawn out (FIG. 2L, 2K, 2N) and pointed like the tips of the apical spines. As a result, when the conidia are first observed separate from the conidiophore, it is difficult to determine where and how they are borne. In mature and old spores this basal spine may readily break off (FIG. 1K), leaving the spore base blunt or rounded. This extension of the basal cell is not universal, however, since many conidia may be found in which it has undergone but little modification.

The conidia germinate readily in water and in standard culture media in 2 to 10 hours. Sometimes they may even germinate on the conidiophores before reaching maturity. All cells appear capable of germination, and it is not at all uncommon to find germ tubes growing out in all directions from the conidia. However, very few spores have been observed in which the tip cells of the spines were germinating. Successive stages of germination in a hanging drop culture are shown in figure 3. In the majority of

mature and old spores the cells tend to swell up and become somewhat vesicular before the germ tubes appear (FIG. 3B); and as a consequence the conidia increase considerably in size and volume. When the germ tubes first appear they may be fairly slender, but after attaining some length they increase in diameter, branch, and become septate. In a large number of conidia observed the first cell of the germ tube became greatly enlarged and vesicular like the cells of the spores (FIG. 3C). The latter, however, were still clearly distinguishable by their thick walls. In the process of germination the refractive globules may become broken up, and some of them wander out into the germ tubes. In other cases they may remain behind and gradually disappear, suggesting perhaps that they are reserve food which is used up in initial growth.

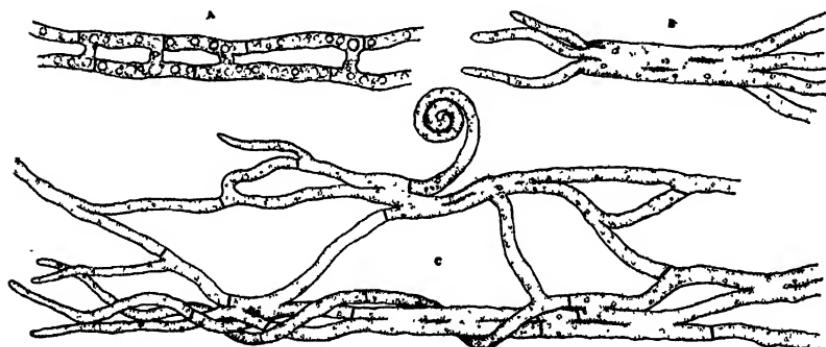


FIG. 4. Variations in hyphal anastomosis.

The mycelium is hyaline, septate, and highly branched and at maturity contains numerous refractive globules of various sizes like the conidia (FIG. 4D). In old cultures, particularly on potato dextrose agar, these bodies may often have a greenish yellow color, as Chodat has already described. This coloration together with the fact that the mycelial cells may sometimes break apart and appear as single individuals doubtless accounts partly for the inclusion of this fungus among the algae. Anastomosis is fairly frequent in hanging drop cultures, and is also common in nature and agar cultures. In figure 4 are shown some of the more unusual cases observed in hanging drops. It may be intercalary by the formation of short side branches (FIG. 4A), terminal (FIG. 4B), or lateral (FIG. 4C). In some hanging drop cultures anastomosis

has been so abundant as to form a network of filaments, and in other instances, an almost dendritic habit of growth. This may be partly due to the unusual conditions in the hanging drop chambers, since hyphal fusions have not been found to be so pronounced in nature. The diameter of the mycelium varies considerably and seems to depend to some extent on age and degree of anastomosis. Variations of from  $3.5\ \mu$  to  $10\ \mu$  have been observed in agar cultures.

#### CULTURAL CHARACTERISTICS AND PATHOGENICITY

*Tetracladium Marchalianum* has been grown on a large number of synthetic agars including corn meal, pH 6.0; potato dextrose, pH 5.5; Sab. dextrose, pH 5.7; prune, pH 5.7; Russell double sugar, pH 7.3; and Saccharosemannitol, pH 7.35. It grows slowly and well on all of these media, but fastest and best perhaps on corn meal and potato dextrose agars. On these latter two, colonies 7.5 cms. in diameter may be formed within a month. On cornmeal agar the colonies are symmetrically circular in outline and with well marked concentric rings, light pearl grey in color, smooth and comparatively thin; while on potato dextrose and Sabouraud's dextrose agars they are strikingly different: thicker, heavier, more felt-like with prominent concentric rings and conspicuous ridges and depressions radiating from the center. The surface of the colonies appears quite mealy, and the center is considerably elevated. In one-month-old colonies the central zone tends to be pinkish grey, then follows a mealy grey bank surrounded by a wider slightly lemon yellow zone, which finally fades out at the edge to a light pearl gray ring. These faint variations in color together with the mealy surface, radiating ridges, and depressions make a totally different picture from that presented on corn meal agar culture.

On saccharose-mannitol and Russell's double sugar agars with phenol red as indicators, growth is much slower; month-old colonies rarely being more than 2.5 cms. in diameter. They are generally heaped up or elevated in the center and markedly ridged. Acid production is quite pronounced and rapid, since within 12 to 18 hours the color of the agar has changed to amber yellow. On prune agar the colonies are again quite characteristic. The hyphae

appear to be more densely aggregated into strands, which radiate from the center and become markedly branched, frayed, fluffy, and lace-like at the edge. As a result the outline of the colony may become somewhat irregular. Concentric rings are none the less conspicuous, and the color of the colony as a whole is a dirty grey, becoming slightly lavender grey in older cultures. Growth is somewhat more rapid than on the double sugar agars; many month-old colonies being 3.5 cms. in diameter. Variations in habit and rate of growth naturally occur in all these agars, but the descriptions given above are fairly characteristic.

This fungus also grows well in liquid media. All of the above named agars were diluted to liquid consistency with distilled water, sterilized in flasks, and inoculated with small cubes of agar in which the mycelium only was growing. These cubes sank at once to the bottom, and within a fortnight vigorous hemispherical colonies had developed around them. The colonies continued to grow until the flasks were filled, and on reaching the surface spread out into a flat mat and formed abundant conidia. So far conidia have only been observed on the surface of such media.

As to the pathogenicity of the *Tetracladium Marchalianum* very little definite is known. De Wildeman, Chodat, Saccardo, Lindau, and Lowe describe it as a saprophyte, but Putteman, according to De Wildeman (1920) regards it as a dangerous parasite to certain vegetables, particularly leeks. Also Soraur has found it in Berlin on decaying hyacinth buds. Preliminary attempts have been made at Columbia to inoculate *Isoetes lacustris* in battery jars with rich spore suspensions, but all results have so far been negative. It has also been tested on detached fruits and vegetables such as oranges, apples, bananas, potatoes, etc., without positive results.

#### DISCUSSION

It is quite obvious from the above description that *Tetracladium Marchalianum* De Wild. is a fungus and should be clearly separated from any of the algae genera and species with which it has been related in the literature. While most mycologists have regarded it in this light, many algologists have confused it with old, doubtful, and poorly defined genera of the algae, with the result that the latter have acquired the stigma of uncertainty and

have been excluded from many textbooks of algology. Neither *Astrothrix*, *Phycastrum*, nor species of *Cerasteria* are included by Oltman (1922), Printz (1927), and Fritsch (1935), while Brunnthaler (1915), West (1916, 1927), and Smith recognize *Cerasteria* only in a limited sense. With the view of presenting this problem more concisely, I have brought together in figure 5 various illustrations of the questionable genera and species with which this fungus has been associated in the literature as well as the early figures of *T. Marchalianum* itself.

The genus *Astrothrix* with the species *microscopica* was established by Kützing in 1843, and as is shown in figure 5A the thallus consists of fairly short, branched filaments with oval and elongated cells or buds at the apices. In this latter respect it is strikingly like a monilioid fungus instead of an alga, which doubtless led Ström (1923) to the opinion that Kützing's figures relate to one of the lower fungi. Except for the habit of branching of the small thalli there is very little similarity, in the author's opinion, between this species and *T. Marchalianum*. On the other hand, *Astrothrix Pertyana* Naeg., as figured and described by Perty (pl. 17, fig. 16) is golden green in color and looks more like a blue-green alga, although the habit of branching is essentially the same as in *A. microscopica* Kütz. Rabenhorst (1868), however, regards *Astrothrix* as a doubtful algal genus and places the three species, *A. microscopica* Kütz., *A. tripus* Braun, and *A. Pertyana* Naeg. among the questionable forms. In 1887 Wolle included this genus in the Oscillarieae section of the Nostocaceae, and established an additional species, *A. Creginii*, from his American material. De Toni (1907) likewise classes *Astrothrix* among the blue-green algae but none the less regards it as a doubtful genus. In 1914 Printz recognized it as a valid genus of the Protococcaceae, but in 1927 he excluded it entirely from the green algae. Ström (1923), on the other hand, while including *A. microscopica* Kütz. among the Myxophyceae, is still doubtful of its algal nature, and believes it is closely related to some of the lower fungi. Finally, in 1925 Huber-Pestalozzia added two new species, *A. sessilis* and *A. spinulosa*, to the genus, but he is likewise doubtful as to its position and relationship among the algae.

*Phycastrum longispinum*, another species with which *T. Mar-*

*chalianum* has been associated in phycological literature, is shown in figures 5B and C. As figured by Perty it is a four-pointed unicellular organism with such deep lobing that no marked central axis or body is present. One of the spines is usually longer than the others (FIG. 5B), while another one drops off very early, leaving only three radiating spines as is shown in figure 5C. Perty figures this species as being deep green in color, which seems to leave little doubt as to its algal nature. In 1867 Reinsch transferred it to his newly created genus *Cerasterias* and changed the species name to *C. longispina*. In the following year Rabenhorst included it in *Polyedrium* and copied Perty's figures for illustration. Wolle likewise included it in this genus; but in 1888 Hansgirg transferred it to *Tetraëdron*, where it has been retained by many algologists, including Brunnthaler and West (1927). De Toni (1889), on the other hand, referred it back again to *Cerasterias*. Finally, in 1914 Printz placed it in the genus *Astrothrix* under the name *A. longispinum* and expressed the opinion that it was probably identical with *C. raphidiooides* var. *inaequale* and *incrassatum* Reinsch (1888). In this manner *T. Marchalianum* came to be involved not only with *Cerasterias* and *Astrothrix* but with *Phycastrum* as well.

In figures 5D-5G is shown *Cerasterias raphidiooides* Reinsch of 1867. Reinsch recognized four forms, *tridens*, *tetradens*, *octodens*, and *obtusa*, on the basis of the shape and number of spines present. Forms *tridens* and *tetradens* are essentially like *Phycastrum longispinum* Perty with the exception of the shorter and more blunt spines. Rabenhorst transferred this species likewise to *Polyedrium* and changed its name to *P. Reinschii*, and since that time has been placed in the genus *Tetraëdron* by Hansgirg, whose classification has been followed by Brunnthaler and West. There seems to be little doubt that *Phycastrum longispinum* Perty and *C. raphidiooides* Reinsch of 1867 are unicellular algae and show no affinity with the fungi, but in *C. raphidiooides* var. *inaequale* and *incrassatum* Reinsch of 1888 we are doubtless dealing with a species of an altogether different group. The outstanding difference, as De Wildeman points out, is the presence of rows of buds or cells in the axis of the spines (FIG. 5H-5J). Such structures are altogether lacking in the former species, according to the figures in

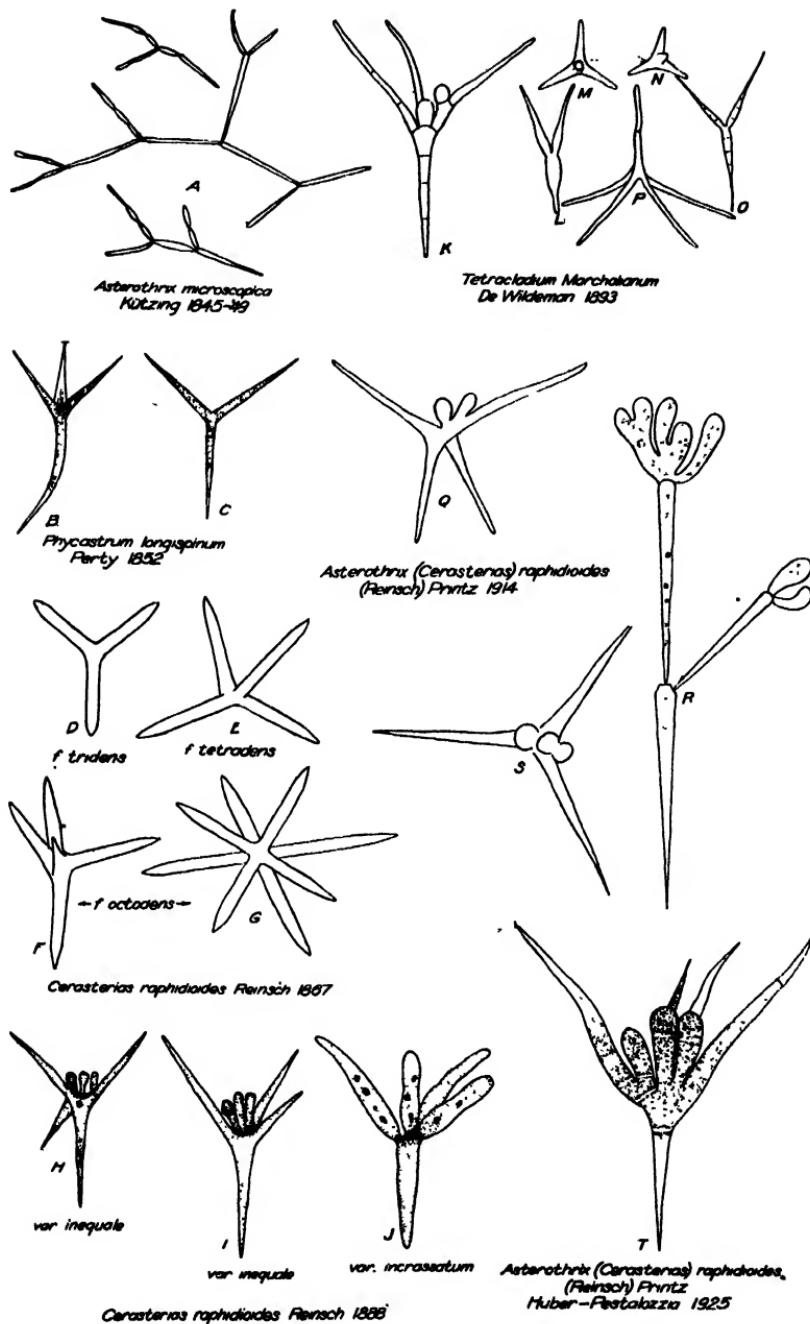


FIG. 5. Algal and fungus species with which *T. Marchalianum* has been confused.

the literature. To anyone who critically examines these and the other figures given by Reinsch and compares them with the conidia of *T. Marchalianum* it becomes at once obvious that vars. *inaequale* and *incrassatum* are identical and represent but variations of fungus spores found by De Wildeman. None the less, De Toni (1889) recognizes it as an alga and Collins (1928) copies the drawing shown in figure 5H as an illustration.

*Tetracladium Marchalianum* as figured by De Wildeman in 1893, is shown in figures 5K-P. A comparison of these figures and those of Perty and Reinsch (1867) leads one to suspect that in this first publication De Wildeman was dealing with a mixed culture. Figures 5K and 5P without question represent fungus spores, but the remaining ones are strikingly similar to the drawings of Perty and Reinsch (1867) and quite probably illustrate algae. It is doubtless the inclusion of such figures, it seems to me, that led algologists to believe De Wildeman was describing algal forms similar if not identical with Reinsch's (1867) *C. raphidiooides*. His subsequent drawings are more distinctive of the fungus, but it seems as if the error, which doomed *T. Marchalianum* to confusion had already been committed.

- The only figure given by Printz in 1914 to illustrate his material of *Astrothrix (Cerasterias) raphidiooides* is shown in figure 5Q. It is almost identical with figure 5H, with the exception that the reflexed spine is on the right side. This undoubtedly represents a fungus conidium, and Printz (1927) is clearly justified, in my opinion, in excluding it from the algae. However, his exclusion of *C. raphidiooides* Reinsch of 1867 is obviously more open to question. Three of Huber-Pestalozzia's drawings of *A. (Cerasterias) raphidiooides* (Reinsch) Printz are shown in figures 5R-T. These are so obviously conidia of *T. Marchalianum* that it is amazing how he could have any doubt as to their real identity. Figure 5R is particularly interesting in that Huber-Pestalozzia interprets it as the budding of a mother thallus to form daughter cells. To anyone who has grown and studied this fungus in culture, it is apparent that this figure really represents a conidiophore with two young conidia in the process of development. The fact that neither Huber-Pestalozzia, Printz, nor Reinsch (1888) ever cultured this organism is doubtless the dominant factor in this

confusing situation. Had they done so, its identity as a fungus would have been immediately recognized.

In conclusion, these observations on *T. Marchalianum* confirm those of De Wildeman in practically every respect, with the exception of classification. While the conidia are multi-cellular and phragmosporous, their outstanding characteristic is one of shape. The spines are usually so elongated and divergent as to give the spores frequently the appearance of a three- to four-pointed star, and for this reason the author believes that this fungus should be retained in the Staurosporeae division of the family Mucedinaceae. Clements and Shear, as noted before, place *T. Marchalianum* in the family Moniliaceae on the basis of their inclusion of the Mucedinaceae with the former family.

As to the algae with which this fungus has been associated in phycological literature, the author agrees with De Wildeman, Smith, and West (1916) that the generic name *Cerasterias* should not be excluded from the algae but retained for such forms as *C. raphidioides* Reinsch 1867, *C. irregulare* Smith 1926, etc., either as a separate genus or a section of *Tetraëdron* as advocated by Hansgirg and Brunnthaler. *Cerasterias raphidioides* var. *inæquale* and *incrassatum* Reinsch (1888) are fungus conidia, and together with *Astrothrix (Cerasterias) raphidioides* (Reinsch) Prinz 1914 are but synonyms of *T. Marchalianum* De Wild. 1893.

#### SUMMARY

1. *Tetracladium Marchalianum* De Wild. has been discovered on dead and decaying leaves of *Isoetes lacustris* growing in the greenhouse tanks at Columbia University.
2. It is without question a fungus which grows readily on ordinary culture media and produces well developed mycelial colonies.
3. The conidia are essentially three- to four-pointed, brush-like, flat, hyaline, multicellular, 22  $\mu$  to 75  $\mu$  in length, filled with refractive globules at maturity, and belong in the Staurosporeae division of the family Mucedinaceae.
4. The mycelium is hyaline, septate, highly branched, rich in refractive globules at maturity, and varies from 2 to 10  $\mu$  in diameter, depending somewhat on age and degree of anastomosis.

5. *Tetracladium Marchalianum* is doubtless an aquatic Saprophyte of wide occurrence. No evidence of parasitism has yet been observed on *Isoetes lacustris*, and it appears to develop only in dead and decaying tissues.

6. This fungus, in the author's opinion, is identical with *Cerasterias raphidiooides* Reinsch (1888) var. *incrassatum* and *inaequale*, and consequently the name *T. Marchalianum* should supersede it, since it is confused with the algal genus bearing the same name.

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# THE TERMINOLOGY OF THE CRYPTOCOCCI WITH A NOTE ON CRYPTOCOCCUS MOLLIS<sup>1</sup>

RHODA W. BENHAM

(WITH 2 FIGURES)

The question of the correct terminology for those groups of fungi which resemble the yeasts, in that the vegetative body consists of round or oval budding cells, but differs from the true yeasts in not forming ascospores, has long troubled those working with these organisms. Many names have been suggested but none has been uniformly accepted. The two names which are most commonly employed are *Cryptococcus* and *Torula*. The latter is used most extensively by botanists and students of the industrial yeasts, the former by dermatologists.

The name *Torula* was first used for these yeast-like forms in 1838 by Turpin (1). Confusion resulted, however, as the name *Torula* had been given by Persoon in 1801 (2a, b) to one of the Fungi Imperfecti belonging to the Dematiaceae. Persoon's *Torula* is characterized by short dark hyphae with chains of globose to oblong conidia which readily fall apart. Nevertheless, Pasteur (3) and Hansen (4) in their later studies continued to use the name *Torula* for yeast-like forms without spore production, but having different intensities of alcoholic fermentation. In an attempt to remedy the confusion caused by the use of the name *Torula* for two quite different types of fungi, Berlese as quoted by Ciferri (5), in 1894 proposed to substitute for the name *Torula* Turpin (Pasteur-Hansen) the term *Torulopsis*, leaving the name *Torula* for Persoon's genus. This name was accepted by Saccardo (6) but it has never come into general use. Will (7) in 1916 distributed the non-spore forming, non mycelial ferment

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in two genera, *Torula* and *Eutorula*. Ciferri (5) in 1925 placed these forms in the two genera *Torulopsis* and *Eutorulopsis*, corresponding respectively to Will's *Torula* and *Eutorula*.

Clements and Shear (8) list the names *Torula* (Turpin), *Eutorula*, *Torulopsis*, *Eutorulopsis* and *Cryptococcus* as synonymous and give preference to *Torulopsis*.

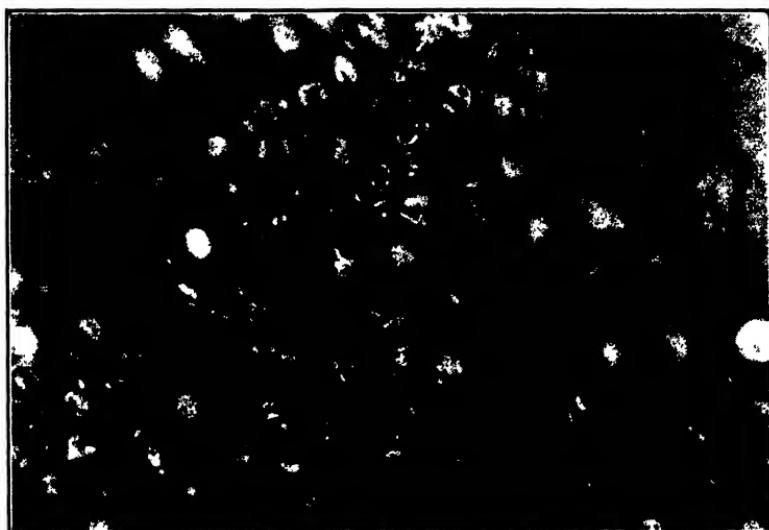


FIG. 1. Photomicrograph of *Cryptococcus mollis* from herbarium specimen,  $\times 2000$ .

The name *Cryptococcus* was first used by Kützing (9) in 1833 for a microorganism which he found on moist window panes and which he classified among the algae. Vuillemin (10) in 1901 adopted the term for pathogenic yeast-like fungi which develop no ascospores. He gave the name *Cryptococcus hominis* to the fungus which Busse and Buschke had described as a *Saccharomyces* or "Hefe" without suggesting any specific name. Guilliermond (11) follows him in placing the pathogenic yeasts which do not sporulate in the genus *Cryptococcus* and similar non-pathogenic forms in the genus *Torula*, although he states they may all be considered part of the latter genus.

Ota (12) follows these authors in retaining the name *Cryptococcus* for non-mycelial, non-spore forming yeast-like fungi. He gives *Cryptococcus hominis* as an example of a pathogenic *Cryp-*

*tococcus*. Castellani (13) described a number of species of *Cryptococcus* and in his Adolph Gehrmann lectures followed Vuillemin and Guilliermond in the use of *Cryptococcus* for the pathogenic and *Torula* for the non-pathogenic forms.

The majority of medical writers have followed this usage. On the other hand, Stoddard and Cutler (14) gave the name *Torula histolytica* to a fungus causing meningitis and this name has obtained wide acceptance although it seems certain (15) that their fungus is identical with that discovered by Busse and Buschke and previously named by Vuillemin *Cryptococcus hominis*. Recently the terms *Eutorula* and *Torulopsis* appear occasionally in descriptions of pathogenic forms. Castellani (16) refers to *Cryptococcus hominis* as *Torulopsis hominis* in a recent paper and Lodder (17) prefers *Torulopsis* as the generic name for this fungus.

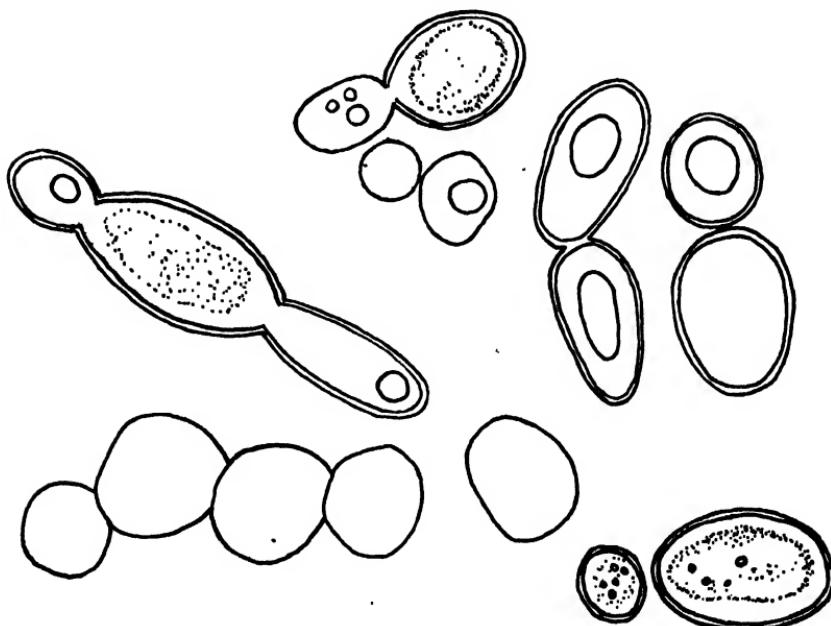


FIG. 2. Camera lucida drawing of *Cryptococcus mollis* from herbarium specimen,  $\times 4000$ .

The use of so many different names for the same fungus has led to regrettable confusion, and a general acceptance of one term is desirable. Priority is the most secure ground for deciding on

the correct term but some authors would give consideration to common usage. Dr. C. L. Shear in a recent publication writes as follows: "Fortunately, thus far, no very widespread and serious attempts have been made to change our fungus names on a strict priority basis, and we know of nothing that would interfere more with the advancement and popularization of systematic mycology than a general attempt to apply this plan to the names of fungi, as it would result in a change of a great many of our best known names of genera and species. The names at present in general use should be conserved if we are ever to have a reasonably uniform and stable nomenclature." This idea seems especially important in medical mycology where many names which are nomenclaturally incorrect have become familiar by common usage.

In the present instance it should not be difficult to determine the correct name on grounds of priority. As has been stated, *Torula* (Persoon 1801) was a dematiaceous fungus and does not belong to this group. *Cryptococcus* (Kützing 1833) takes precedence over *Torula* (Turpin 1838) and the other generic names discussed. The question would seem to depend then on whether the micro-organism which Kützing described belongs to this group.

The objection has been raised that Kützing considered his *Cryptococcus* an alga. His description of the original species *C. mollis* is as follows: "Globuli mucosi hyalini non colorati microscopici in stratum indeterminatum mucosum facile secedens sine ordine aggregati. An feuchten und schmutzigen Fenstern." In his 1849 publication he lists thirteen species as follows: *C. nebulosus*, *natans*, *Rhei*, *Valeriana*, *mollis*, *inaequalis*, *roseus*, *carneus*, *coccineus*, *brunneus*, *vernicosus*, *Cerevisiae* and *Vini*. From the descriptions most of these species belong to the yeast-like fungi. None of them was a true alga. *Cryptococcus Fermentum* seems with certainty to belong to the yeast group and is generally accepted as a synonym of the common yeast *S. Cerevisiae*. In his 1845 publication in *Phycologia germanica*, Kützing describes *C. Fermentum* as follows: "*C. Fermentum* (Hefe) Kugelchen 1/700-1/600" Gross, elliptisch, mit 1-2 punkten in innern" and in the list of thirteen species, *C. Fermentum* is given as a synonym for *C. Cerevisiae*. Saccardo regarded the term *Cryptococcus* as

a synonym of *Saccharomyces*. The name is not used by algologists.

In Kützing's 1833 publication, Decas III, is to be found together with the description of the species *Cryptococcus mollis*, an herbarium specimen. This publication is to be found at The New York Botanical Garden. Through the kindness of Dr. J. H. Barnhart and Dr. M. A. Howe, I have been able to examine some of this material.

A little of the dried specimen was scraped off in water and later mounted in glycerine for study. The material is very well preserved and one can note a number of organisms present, including some bacteria. There is nothing in the material that would suggest an alga. By far the most abundant organism present is in the form of oval cells which resemble the yeasts. There is definite evidence of budding as may be seen in figures 1 and 2, though this was not mentioned in Kützing's description. The buds often remain attached forming chains of cells, but nothing like a true mycelium is seen. This form then resembles very much the *Cryptococcus* forms, and it seems clear that the name was given to yeast-like fungi. Although it would be impossible to identify *Cryptococcus mollis* with certainty, it seems evident that it was not an alga but a yeast-like fungus similar to the forms which have since been called *Cryptococcus*, *Torula*, *Eutorula* and *Torulopsis*. This would seem to establish the priority of Kützing's term.

So much for priority. Usage is less easy to determine, but the term *Cryptococcus* seems at least as familiar as *Torula* and is more commonly employed by medical mycologists than the latter term. The names *Torulopsis* and *Eutorula* have no wide currency. It would seem then that the generic name *Cryptococcus* deserves preference on grounds both of usage and of priority.

In conclusion it is suggested to emend Vuillemin's definition of the genus *Cryptococcus* somewhat as follows:

Order Moniliales: Family Pseudo-saccharomycetaceae, genus *Cryptococcus*.

Unicellular fungi consisting of round or ovoid cells occasionally in chains but never forming a well-defined mycelium. Repro-

duction by one or more buds; no ascospores. Growth on artificial media in pasty or dry colonies, white or colored.

It is further recommended that the generic names *Torula*, *Torulopsis*, and *Eutorula* be discarded as being synonymous with *Cryptococcus*.

In view of the fact that the original species *Cryptococcus mollis* is of such doubtful identity we would suggest *Cryptococcus hominis* Vuillemin as a representative species. It may be defined as follows:

Cells round to oval 3–8  $\mu$  in diameter, usually 4–5  $\mu$ . Contents granular with lipoid globules, wall distinct and surrounded by a clear zone of capsular substance. Buds usually single. In tissues of host occur scattered through a clear homogeneous substance forming a zoöglia-like mass. Giant colonies circular with a smooth glistening surface and mucoid consistency, color white or tan, or deep brown. Frequently pathogenic for man, rats and other animals. Habitat: Tissues of infected humans, horse and cheetah, skin and intestinal canal of normal humans.

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# CYTOLOGICAL STUDIES OF THE TREMELLACEAE<sup>1</sup> III. SEBACINA<sup>2</sup>

R. M. WHELDEN

(WITH 3 TEXT FIGURES)

The genus *Sebacina* comprises small usually very inconspicuous fungi apparently wide spread in occurrence, particularly in North America. While it has been split into several genera, Rogers (4) has pointed out that such a separation is unnecessary and has reduced the segregates to subgeneric rank. The writer has followed him in this, considering all resupinate tremellaceous fungi with waxy consistency to be species of *Sebacina*.

Three species were selected for intensive study; these were *Sebacina deminuta* Bourd. (*Bourdotia deminuta* Bourd.), *S. fugacissima* Bourd. & Galz.,<sup>3</sup> and *S. epigaea* (Berk. & Br.) Bourd. & Galz. The first species forms extremely thin greyish patches on the bark and wood of a partially decorticated fallen poplar trunk. On drying, these patches shrink to a faint inconspicuous glair easily overlooked. This species is characterised by having in the hymenial layer elongate hyphal tips, the gloeocystidia, whose protoplasm contains much yellowish "resinous" substance. *S. fugacissima* has brownish fruit-bodies somewhat more conspicuous than those of *S. deminuta*. In these fruit-bodies occur cystidia, enlarged hyphal tips devoid of protoplasmic content, projecting up through the hymenium. The third species studied, *S. epigaea*, has much larger light brown fruit-bodies growing over the ground,

<sup>1</sup> Contribution No. 134 from the Laboratories of Cryptogamic Botany at Harvard University.

<sup>2</sup> The writer is pleased to acknowledge his gratitude to Dr. D. H. Linder, who collected and preserved in the field much of the material used in the present study. The writer also wishes to thank Prof. W. H. Weston, Jr., for his helpful criticisms during the writing of this paper.

<sup>3</sup> Specimens of this fungus were submitted to Prof. G. W. Martin, who very kindly determined it as *S. fugacissima* Bourd. and Galz.; for this determination I am indeed grateful.

fallen leaves and debris. In these fruit-bodies neither cystidia nor gloeocystidia were present at any time during development.

#### HISTORICAL BACKGROUND

Comparatively little work has been done on the cytology of this group. In 1902 Maire (2) briefly described the development of the basidia of *S. effusa* Bref. In the young basidium the two nuclei with densely staining nucleoli and rather small karyosomes united to form the rapidly enlarging fusion nucleus in which the karyosomes were "en forme de longs filaments chromatiques, très fins, pelotonnés lâchement." When the basidium was fully mature there appeared extending between definite centrosomes a transversely oriented spindle at the center of which was a chromatin mass which separated into two groups each having two chromosomes. Subsequently the two daughter nuclei, separated by a longitudinal septum, divided again in a way very much like the first division, after which each nucleus migrated into a spore. On germination of this spore another nuclear division might occur.

In 1924, Neuhoff (3) verified in the main Maire's work, although he could not determine the number of chromosomes, due to their minute size. Neuhoff also described, in *S. calcea* (Pers.) Bres., the history of the fusion nucleus, in which he saw two faintly staining chromatin nets and a tiny black-staining dot giving place to a long persisting spireme. When this broke up into chromosomes, apparently four in number, the nuclear membrane, previously distinct, became vague, while the transversely oriented spindle formed. This first nuclear division was followed immediately by a second, after which there occurred a considerable increase in the volume of the hypobasidium. Only when this increase was complete did the usually obliquely directed septa divide the hypobasidium into four uninucleate segments.

Kühner, in 1926 (1), noted in the fusion nucleus in *S. gloeocystidiata* Kühner the presence of fine regular chromatin threads and a distinct nucleolus. As division progressed, the nucleolus disappeared while the chromatin threads contracted and became irregular, finally giving rise to an indistinct number of chromosomes massed at the center of the transversely oriented spindle. A second division followed the first, so that eventually there were

formed four nuclei each of which entered a spore. Kühner alone seems to have noted the presence in the hymenium of any other object than basidia. He described the gloecystidia as elongate bodies densely packed with golden-yellow drops of a highly refractive substance. These gloccystidia, in which he saw no nucleus, had helical bands of some substance, which he considered to be vestiges of cytoplasm.

#### MATERIAL AND METHODS

As described in an earlier paper of this series (5), each collection of specimens was divided into two or more portions, each portion was killed in the field by one or the other of the different fixing fluids and subsequently was stained by one of several different methods. As in the genera *Tremella* and *Exidia*, Haidenhain's iron-alum-haematoxylin gave by far the most satisfactory nuclear figures.

#### DESCRIPTION

The same general structure is revealed in sections cut through the center of the fruit-bodies of all species of *Sebacina*. Over the surface of the substratum there spreads a layer of closely interwoven hyphae, which in *S. fugacissima* is so dense as to render difficult the determination of individual hyphae. Above this basal layer arises a much looser stratum of hyphae extending into the superficial hymenium. In *S. deminuta* the subhymenial portion of the fruit-body is rarely more than 20  $\mu$  thick, in *S. fugacissima* it ranges up to 100  $\mu$ , while in *S. epigaea* the thickness is from 600 to 700  $\mu$ . Dried material brought into the laboratory and wetted seldom yields measurements equal to those obtained in the field.

In all species here studied the short segmented hyphae in the lower region seemingly are nearly devoid of protoplasm, while the more loosely arranged hyphae of the upper layer have longer, usually binucleate segments, filled with protoplasm (FIG. 1: 1). Near the surface these frequently end in long multinucleate tips, particularly in *S. epigaea* (FIG. 3: 25-28). No clamp connections nor interhyphal fusions were seen in any part of the fruit-bodies.

In the hyphal tips, often much branched, nuclear divisions are frequently observed in abundance. The small size of the nuclei, from 0.5 to 1  $\mu$  in diameter, makes it very difficult to determine

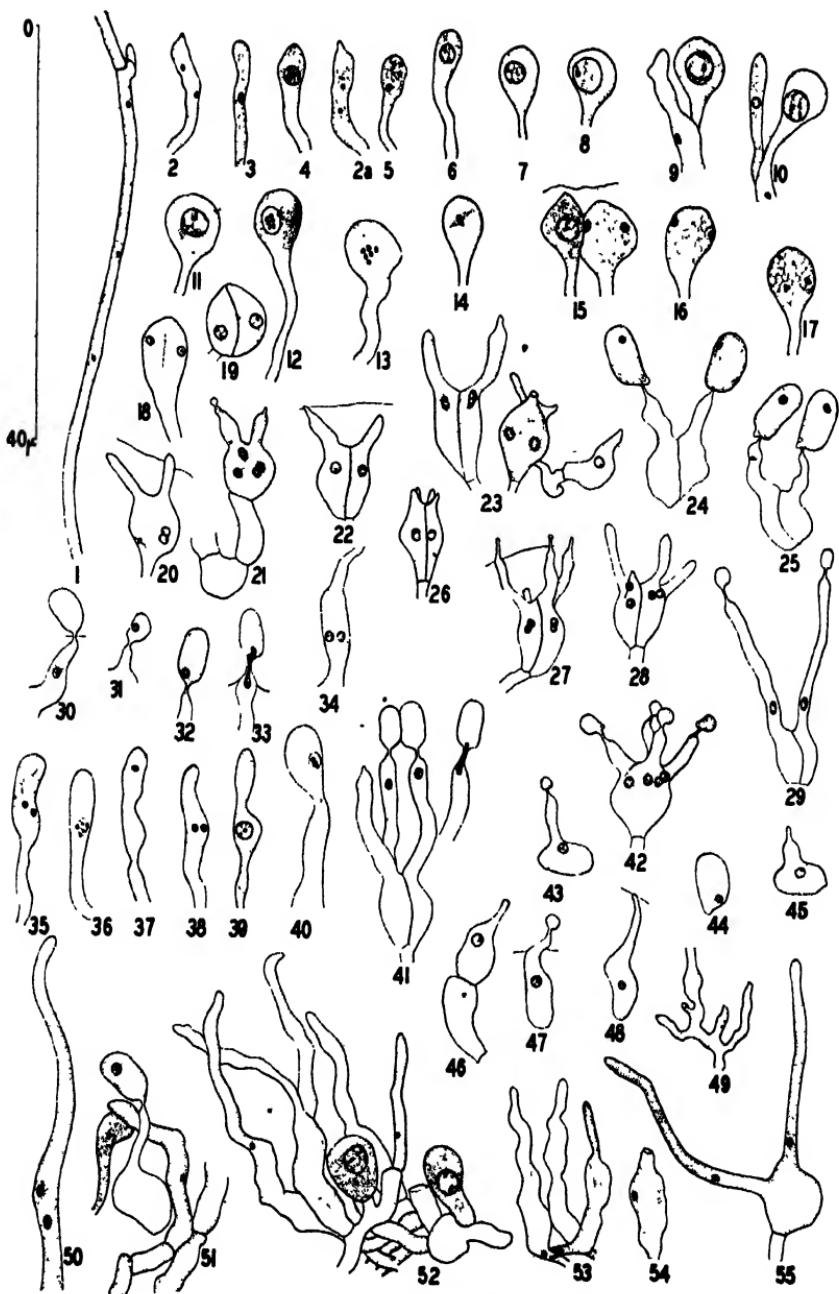
**SEBAGINA DEMNUTA**

FIG. 1.

the number of chromosomes. Three chromosomes are always seen; frequently there is observable near the chromosomes a minute object, which under certain conditions gives an impression of four chromosomes in the nucleus (FIG. 3: 28, lowest nucleus).

The youngest basidium initials, always binucleate, differ from the hyphal tips in having slightly denser protoplasmic contents, and a somewhat greater diameter (FIG. 1: 2; FIG. 2: 1, 2; FIG. 3: 1, 2). Even before the two small nuclei fuse, enlargement of the hypobasidium is quite noticeable, particularly in *S. deminuta* (FIG. 1: 3, 5). This enlargement becomes much more pronounced subsequent to the fusion of the nuclei and continues until the fully mature hypobasidium obtains (FIG. 1: 4, 6-7; FIG. 2: 3-9; FIG. 3: 3-7). The dimensions of the latter are extremely variable, but average as follows: in *S. deminuta*, they are subspherical bodies about 5 to 6.5  $\mu$  in diameter (FIG. 1: 8-11); in *S. fugacissima*, slightly larger bodies averaging  $8 \times 6.3 \mu$  (FIG. 2: 10); in *S. epigaea* much larger and often more irregular objects varying from  $10 \times 7 \mu$  to  $15 \times 8 \mu$  or even larger (FIG. 3: 8, 9).

During this enlargement of the hypobasidium, characteristic changes occur in the fusion nucleus which has increased in size correspondingly. In the species of *Sebacina* here studied fusion of the nucleoli is often delayed until considerable enlargement of the fusion nucleus has occurred (FIG. 1: 4). From the beginning of this enlargement the chromatin has been aggregated into definite linear masses which are located near the surface of the nucleus. While these masses are not as distinct as are those in the fusion nuclei of *Tremella* or *Exidia*, the number seems quite definitely to be six (FIG. 2: 11), a fact borne out when contraction of the masses has given rise to the six very small chromosomes. It is interesting to note that, particularly in *S. epigaea*, there appears to be irregularity in the time of contraction of these prochromosomes, so that chromosome-like bodies appear on the surface of the very large fusion nucleus (diam. 5  $\mu$ ) while most of the chromatin remains in the very long patches (FIG. 3: 9). In this species also the number of chromosomes finally formed is difficult to determine, due to the tendency of the chromosomes to clump, and to the apparent fact that the individual chromosomes are unequal in size. Favorable cases indicate the number to be six (FIG. 1: 12, 13; FIG.

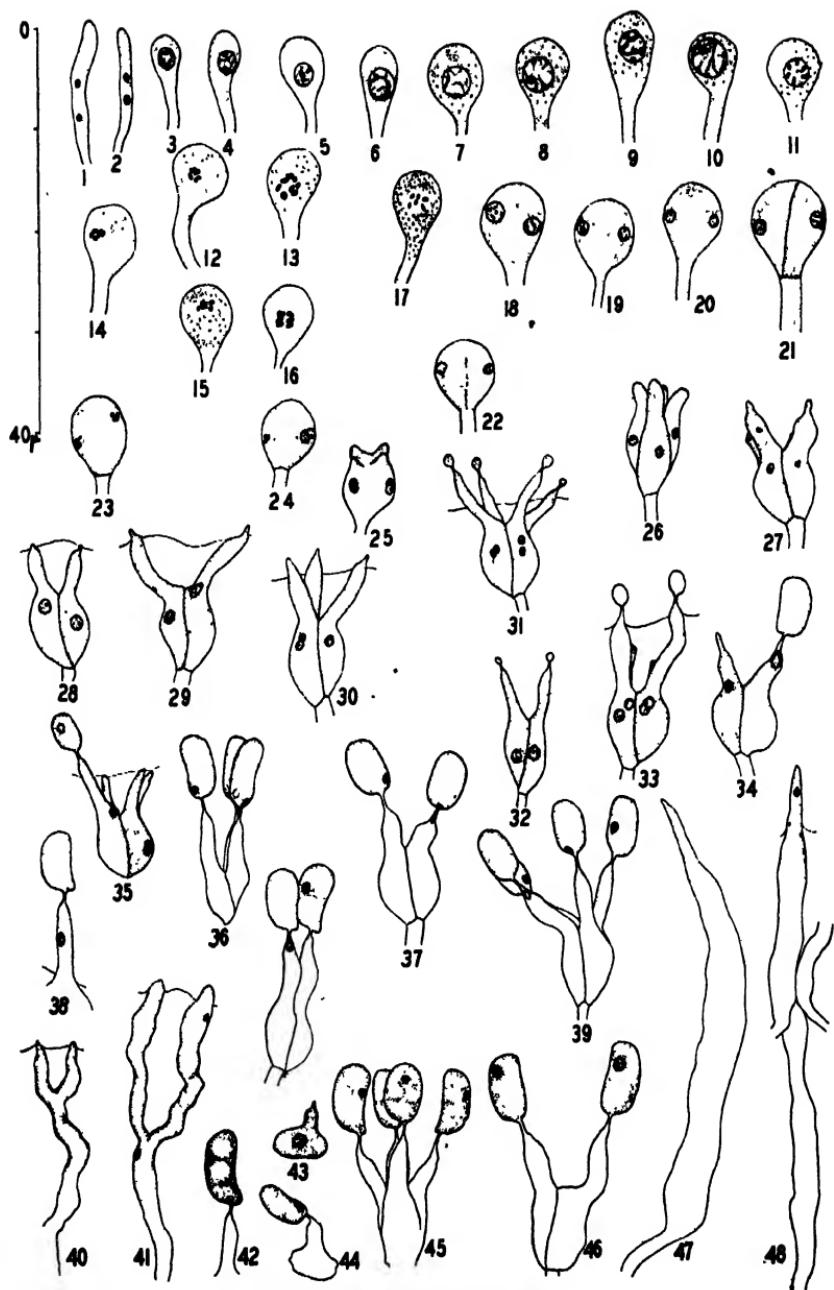
*SEBAGINA FUGACISSIMA*

FIG. 2.

2: 12-17; FIG. 3: 11-13). This conclusion is supported by observation of dividing nuclei in the actively growing vegetative hyphae of young fruit-bodies (FIG. 3: 25-29); which show the haploid number as three.

In the fusion nucleus, the chromosomes lie clumped quite closely on the very vague transverse spindle (FIG. 1: 4); when the chromosomes are formed, both nucleolus and nuclear membrane have disappeared. Although at each pole of the spindle, a suggestion of a dark staining object appears, no definite centrosomes can be distinguished. The two daughter nuclei, quite variable in size in each of the three species, are eventually formed far apart near the wall of the hypobasidium, in which they are usually situated at about the same level (FIG. 1: 15, 16; FIG. 2: 18-20; FIG. 3: 14). The second nuclear division, of one or both nuclei, occurs immediately after the first, resulting usually in four nuclei from 0.5-1  $\mu$  in diameter, that lie near the lateral surface of the hypobasidium (FIG. 1: 17, 20; FIG. 2: 23-25; FIG. 3: 15).

In general, the time of septum formation is not correlated with the divisions of the nucleus. At the time of the first, and often the second of the nuclear divisions, the basal septum cuts off the hypobasidium, frequently several  $\mu$  below the enlarged portion, notably in *S. epigaea* (FIG. 1: 18; FIG. 2: 22; FIG. 3: 11-15). The longitudinal septa, frequently directed obliquely in the hypobasidium, are formed soon after the nuclear divisions are completed (FIG. 1: 18, 19; FIG. 2: 21, 22). The cytoplasm of the mature hypobasidium, which is commonly either two or four celled, very rarely three-celled, has become increasingly vacuolate.

Only rarely do the epibasidia begin to form before the nuclear divisions are completed. In young fruit-bodies of *S. deminuta*, the epibasidia, which first appear as minute bulges from the apical surface of the hypobasidium, grow rapidly to short, irregular, somewhat obconical structures, generally spreading at a considerable angle one from the other (FIG. 1: 22). The tip of each epibasidium gradually narrows to form a slender sterigma about 1  $\mu$  long at the end of which the spore develops (FIG. 1: 21, 22, 27). In the older fruit-bodies the much longer epibasidia grow directly out to the surface of the "jelly" (FIG. 1: 29, 41). In *S. fugacissima*, the very coarse epibasidia, usually from 2 to 2.6  $\mu$  in diam-

eter, grow outward in rather irregular fashion to a length of 6 to  $8\mu$  then abruptly narrow to the very slender sterigmata (FIG. 2: 26-30). In *S. epigaea*, the epibasidia, strikingly unlike those of the other two species here described, first appear as coarse tubes, from 2.0 to  $2.5\mu$  in diameter, arising in a compact group from the apex of the hypobasidium (FIG. 3: 16). As the epibasidia grow to their maximum length of 50 to  $80\mu$ , the diameter gradually increases to 3 to  $3.5\mu$ . Throughout this growth the two, or more frequently four, epibasidia are nearly parallel (FIG. 3: 22-24, 31). On reaching the "jelly" surface each epibasidium usually narrows abruptly to form the relatively large sterigma  $0.7\mu$  in diameter and 2 to  $4\mu$  long (FIG. 3: 18, 19); less commonly the epibasidium protrudes 5 to  $7\mu$  above the "jelly" surface before narrowing to the sterigma (FIG. 3: 32).

Nuclear migration in all three species usually begins at an early stage in the formation of the epibasidia. In its migration from the hypobasidium through the epibasidium, the nucleus undergoes very slight elongation until near the base of the sterigma (FIG. 1: 21, 23, 28; FIG. 2: 29, 31; FIG. 3: 22-24). While the nuclei of any basidium generally migrate simultaneously, at times, the greatest irregularity obtains, one nucleus being far behind the others (FIG. 3: 31) or, in other cases, one nucleus being in the spore before the others have left the hypobasidium (FIG. 2: 35). Great elongation of the nucleus necessarily occurs during its passage through the slender sterigma (FIG. 1: 41; FIG. 2: 36, 37). Once within the spore the nucleus immediately becomes spherical, and moves to its final position in the rapidly maturing spore (FIG. 1: 44; FIG. 2: 42, 45, 46; FIG. 3: 34, 37-40).

A tiny spherical enlargement at the apex of the sterigma is the first appearance of the spore: this tip, which appears as nuclear migration begins (FIG. 1: 27; FIG. 2: 31, 32; FIG. 3: 17), rapidly enlarges, remaining spherical and usually without dense protoplasmic contents, until the nucleus has migrated almost to the apex of the epibasidium (FIG. 1: 29, 30, 42; FIG. 2: 33; FIG. 3: 20, 21, 30). Then very definite elongation of the spore occurs, with continued increase in diameter, until the dimensions of the mature spore are attained (FIG. 1: 31-33, 41; FIG. 2: 36-39, 42, 45, 46; FIG. 3: 33, 37, 39).

Subsequent to the migration of the nucleus from the hypobasidium into and through the epibasidium, increasing vacuolation occurs, soon resulting in an absolutely empty basidium, as for example FIG. 1: 24, 25, 41; FIG. 2: 36-39, 45-46; FIG. 3: 22-24; in the very large basidia of *S. epigaea* this disappearance of protoplasm is especially noticeable, the withdrawal following close after the migration of the nucleus. In this species, also, complete collapse of the empty basidia soon occurs, rendering extremely difficult accurate tracing of the entire structure.

Soon after its maturity, the spore germinates by means of a single short lateral germ tube, which ends abruptly in a slender sterigma at the tip of which is formed a single secondary spore quite like but smaller than the primary spore (FIG. 1: 43, 45; FIG. 2: 43, 44). Into this secondary spore the single nucleus migrates.

In *S. deminuta*, one frequently finds, particularly near the outer margin of the fruit-body, irregular, spherical to fusiform terminal cells each containing a single nucleus about  $1\text{ }\mu$  in diameter (FIG. 1: 23). From the apical end of this cell a single tube from  $2-7\text{ }\mu$  long develops either upright to the substratum or extending out over its surface (FIG. 1: 46, 48). The end of this tube narrows to a slender sterigma at the end of which there forms a spore entirely like the normal basidiospore (FIG. 1: 47, 51).

In addition to the reproductive cells just described, there are in the fruit-bodies of *Sebacina* other objects which are distinct from the basidial initials in having a less dense protoplasmic content, and which also in many cases show a characteristic early branching. These structures, cystidia, gloeocystidia, paraphyses and dendrophyses, show at first little that is distinctive. They first appear as erect hyphal tips whose two nuclei occupy a position near the center, and finally side by side (FIG. 2: 34, 35, 38), if indeed they have not disintegrated prior to this. Further development shows definite differences distinguishing these objects. Growth of the gloeocystidium continues until it becomes an irregular tube  $8-16\text{ }\mu$  long and  $2.0-2.5\text{ }\mu$  in diameter, in which the protoplasm generally becomes progressively thinner while there accumulate increasing quantities of oil-like drops (FIG. 1: 37). This thinning of the protoplasm becomes more pronounced with further enlargement of the gloeocystidium, which now develops apically a long some-

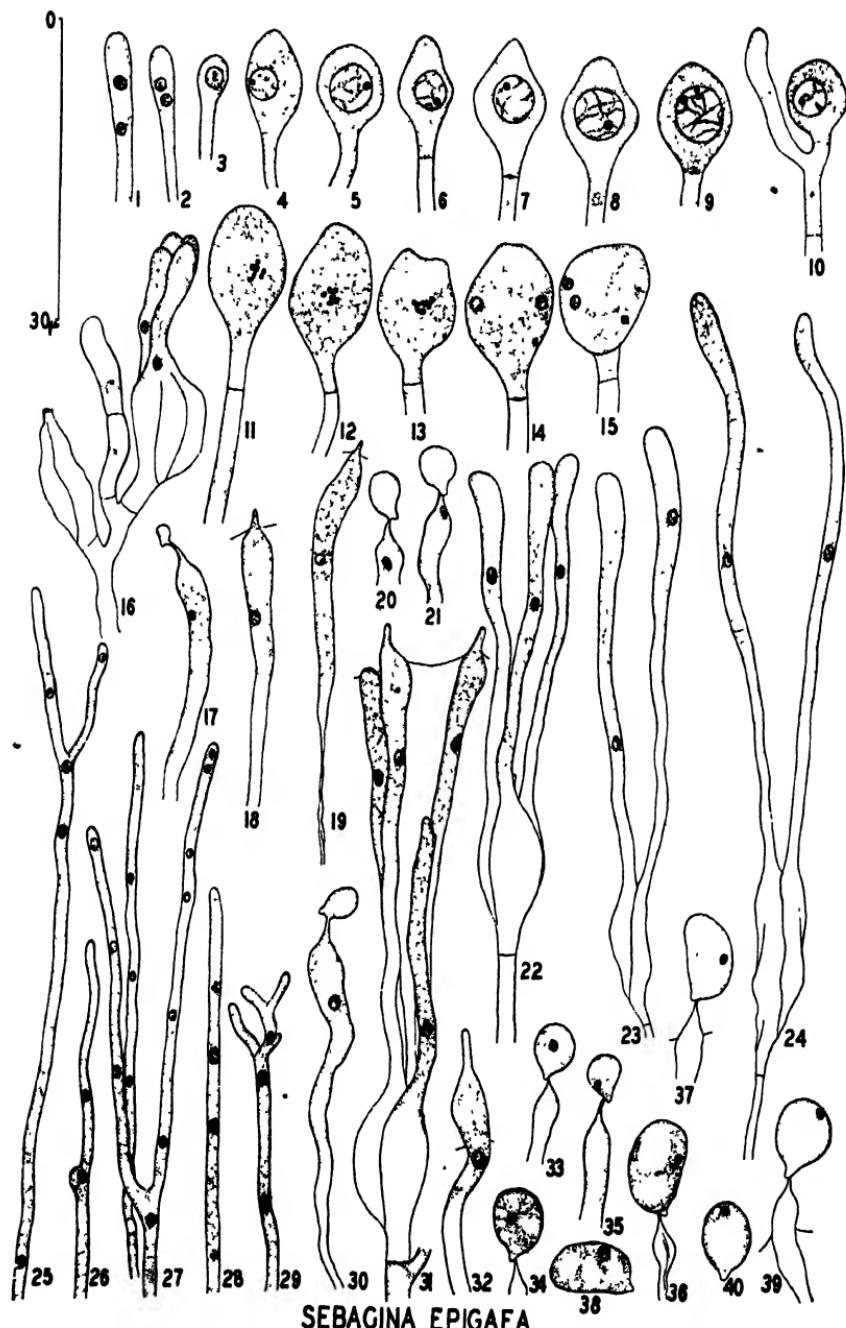


FIG. 3.

what crooked extension, at first seemingly devoid of protoplasm (FIG. 1: 34), but, when 6-7  $\mu$  long, is filled with a uniformly distributed but very thin protoplasmic content (FIG. 1: 52), and many drops of an oil-like substance. Nuclear activities during this apical elongation vary somewhat, but most commonly the two nuclei migrate into the apical portion where first one and then the other disintegrates, after which the protoplasm disappears, leaving a somewhat collapsed tube in which remain vestiges of protoplasm, generally as a faint ring of strand in the region of the junction of the apical portion with the basal (FIG. 1: 53).

Occasionally, even before this apical extension forms (FIG. 1: 37, 39), fusion of the two nuclei takes place, after which the various stages of division of the fusion nucleus (FIG. 1: 36) occur, even up to the formation of somewhat abnormal chromosomes (FIG. 1: 40). Sooner or later, however, the nucleus disintegrates. It is interesting to note that when fusion occurs there is a tendency towards swelling of the containing body, a swelling which is more pronounced the farther the nuclear development proceeds.

Early stages in the development of the cystidia in no way differ from those of gloeocystidia; indeed, were it not that individual species of *Sebacina* seem characterized by one or the other of the two objects, but not both, it would be impossible to distinguish them in early stages. Gloeocystidia long possess contents of "resinous" nature, whereas cystidia early become extremely vacuolate while at the same time they increase tremendously in size. As this development progresses, the two nuclei come to a position near the tapering tip of the cystidium (FIG. 2: 48), and there disintegrate successively, following which there is a disappearance of all protoplasm (FIG. 2: 47), until eventually a tapering somewhat bent body 25-55  $\mu$  long and up to 5  $\mu$  in diameter is developed. Frequently these objects extend upwards from the lowest stratum of the fruit-body to the "jelly" surface above which they may protrude as slender narrowing tips; in other fruit-bodies two or even three successive strata containing cystidia may be noted. All cystidia soon become devoid of protoplasm, and eventually collapse and are lost in the fruit-body. Certain lateral branches, which form paraphyses are associated with the hypobasidia of *S. epigaea*. These paraphyses become vacuolate even earlier in their develop-

ment than the two objects above described, the protoplasm remaining as a thin lining at the apex and an equally thin band in the middle of the segment. In this band may frequently be seen traces of the disintegrating nuclei (FIG. 3: 10). At times these objects become two or three septate (FIG. 3: 16), but always end as empty somewhat collapsed bodies.

There remains for final consideration those irregularly branched structures which have been called dendrophyses. These also have the same early development, but soon become irregularly much branched (FIG. 1: 49); and keep their dense protoplasmic content throughout their existence. Whether these are distinct objects characteristic of the fruit-bodies of *Sebacina* or merely an early stage in the branching hyphae of the enlarging fruit-body as is the case in fig. 3: 29, has not been determined. They often seem to be distinct objects.

#### DISCUSSION

Corresponding stages of development of the fruit-body in species of *Sebacina* show considerable similarity.

The hypobasidial initials, always binucleate in their earliest stages, form a definite hymenial layer over the exposed surface of the fruit-body. As this gets older, the hymenium becomes thicker and thicker, the basidia forming a dense mass in which no obvious separation of younger basidia as more superficial than the older can be made except in a most general way. In the development of each basidium there occurs an early fusion of the two primary basidial nuclei which seems to incite rapid and pronounced swelling of the hypobasidium. At the same time the fusion nucleus expands correspondingly, with characteristic changes. Most noticeable among these is the early organization of the chromatin into definite discrete strips located at the nuclear surface. These chromatin masses are longer and thinner in *Sebacina* than those noted in *Exidia* or *Tremella*, and seem to be six in number, instead of eight. During this nuclear enlargement, the nucleolus has remained a very conspicuous dark staining body located at the periphery of the nucleus. The mature fusion nucleus is essentially as described by Maire and Kühner, having very evident (Kühner) long slender chromatin threads (Maire) which are not

continuous. The present studies have not supported Neuhoff's observation that the chromatin is aggregated into two faintly staining nets.

The greatest differences exist in the descriptions of developments which occur during the formation of the chromosomes. Maire's observation (2) was that the chromatin becomes gathered into small masses, the protochromosomes, from which the two chromosomes are formed; Neuhoff (3) thought small definite chromosomes, four in number, were massed closely in the center of the hypobasidium; while Kühner (1) found two indistinct masses which he considered to be chromosomes. Present studies indicate that six very small but distinct chromosomes are formed; usually these are so compactly grouped as to render very difficult accurate counting of their number. An added difficulty is the frequent presence of one or two minute bodies with the chromosomes. Coincident with the formation of the chromosomes, the nucleolus and nuclear membrane have disappeared, while the transversely oriented spindle has appeared; this spindle was never a conspicuous object in any sections observed in the present study.

The two daughter nuclei at once take definite form and with no resting period may divide again, although quite commonly one or both of them fails to divide. The small size of these nuclei renders it difficult to follow accurately the changes during this division. It is evident, however, that there are three minute chromosomes which divide equationally, showing that the first division is the reduction division.

As Neuhoff (3) has pointed out the development of the epibasidia is remarkably uniform. The variations which do occur, such as the wide-spreading epibasidia of young fruit-bodies which contrast with the erect closely massed epibasidia formed later seem definitely to result from the increased "jelly" mass through which they grow. Emergence from this "jelly" mass seems to induce the development of sterigmata.

The formation of the septa has occurred at this time and the writer agrees with Neuhoff (3) that they develop very rapidly. Nor have the early stages of nuclear migration offered much of interest. Each nucleus elongates only slightly during the entire period of migration until its passage through the sterigma into

the spore, when, due to the minute diameter of the former, the nucleus necessarily becomes much elongated. However, immediately on entering the spore the spherical form is reassumed. Present studies support Neuhoff's statement (3) that the nucleus enters the spore only when the latter is nearly mature; this may be explained by the fact that nearly all the protoplasm has passed into the spore before the nucleus. There are cases, however, where the spore receives its nucleus before full-size is attained; subsequent increase in size is accompanied by increasing vacuolation of nuclear contents. Spore germination always gives rise to secondary spores entirely similar to the primary; no nuclear divisions have been noted in this germination.

Because of their debatable value as points for generic separations, the various non-basidial objects—cystidia, gloeocystidia, paraphyses, and dendrophyses—which are present in the hymenial layer were particularly noted in the present study. In their early stages these all seem to be identical, except that those objects interpreted as paraphyses seem mostly to be branched from the basidial "stalk." It is extremely difficult to decide when seen in its early development what a given hyphal tip eventually will become. Great increase in size and vacuolation of content accompanied by degeneration of the two nuclei determines the cystidia. Considerable accumulations of "oil"-like contents indicates a gloeocystidium. Present studies have not shown any prominent spiral bands within these such as Kühner (1) has figured and described. Such bands as occur are usually faint and irregular, and are probably vestiges of cytoplasm as Kühner considered them. Final development of gloeocystidia leads to the exhaustion of the "oil"-like content leaving an empty partially collapsed object resembling a small cystidium. Little need be said of paraphyses and dendrophyses, beyond pointing out that many times objects resembling such structures are obviously only stages in the development of basidia or are hyphal growth.

These sterile structures have been used to separate *Sebacina* into several genera, a point discussed by Rogers, who held them to be insufficient for generic separation, but satisfactory for sub-generic distinction. It is difficult to separate the minute *Tremel-*

*lac*, such as *T. Grilletii* Boud., from certain species of *Sebacina*; a resupinate fruit-body does not always obtain, young bodies being often discrete and *Tremella*-like. Texture is equally troublesome, for fruit-bodies of *Sebacina* vary from coriaceous through waxy to gelatinous, depending often on the degree of wetness. Nor do any differences in the structure of the fruit-body appear, unless it be in the sterile structures. So far as the writer's observations go these may help form a means of separation, but in themselves they are not sufficient. Certainly the septate, sterile objects described under *Tremella* (5) have not been observed in *Sebacina*, nor have those objects called cystidia or gloeocystidia been found in *Tremella*. Further critical study of additional species of both genera will be necessary before any definite statements may be made relative to their taxonomic value.

#### SUMMARY

The three species of *Sebacina* studied, *S. deminuta* Boud., *S. fugacissima* Boud. & Galz., and *S. epigaea* (Berk. & Br.) Boud. & Galz., have shown the same uniformity of development as was noted in *Tremella* and *Eridia*. Those binucleate hyphal tips which are to become hypobasidia are to be distinguished at first by a somewhat denser protoplasmic content and by a slightly greater diameter. As development progresses the two nuclei fuse, subsequent to which a pronounced swelling of the hypobasidium occurs, accompanied by an equally pronounced enlargement of the fusion nucleus. From the very first the chromatin material of the latter is definitely aggregated in definite linear patches, apparently always six in number. When the maximum size of the fusion nucleus is reached, contraction of the chromatin masses commences. Coincident with this rapid contraction the nucleolus and also the nuclear membrane disappear. When contraction is complete there are six small chromosomes densely massed near the center of the hypobasidium. These chromosomes separate three and three in reduction division and migrate to the opposite poles of the inconspicuous, transversely oriented spindle. Subsequent to the organisation of the daughter nuclei a second division may occur, either of one or of both nuclei, so that a mature hypobasidium may have two, three, or four small nuclei.

The formation of the septa and the development of the epi-basidia offer little that is noteworthy; the differences in size and direction of growth which have been noted are an aid to separation of species but are not in themselves sufficient therefor. The migration of the nucleus usually does not begin until the formation of the septa is complete. During migration only slight elongation of the nucleus occurs, until it reaches the base of the sterigma, at which time it becomes extremely elongate in order to pass through the narrow tube. Immediately after the passage of the nucleus, the basidium becomes devoid of protoplasm; in the very large basidia of *S. epigaea* this is particularly noticeable. The mature, uninucleate, vacuolate spores germinate by means of a single, lateral germ tube through which the nucleus passes without division into the secondary spore.

Because of their possible value as a means of generic separation the non-reproductive bodies—cystidia, gloeocystidia, paraphyses, and dendrophyses—were particularly noted. All seem to form from binucleate hyphal tips at first not materially unlike hypobasidia. Early in development; however, these sterile tips become more vacuolate and show their characteristic differences; cystidia increase tremendously in size, losing at the same time their nuclei and most of their protoplasm; gloeocystidia become filled with an "oil"-like substance, which, with increasing age, disappears leaving objects similar to but smaller than cystidia; paraphyses early become vacuolate and enucleate; dendrophyses branch irregularly. These bodies become lost in the further development of the fruit-body.

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## EXPLANATION OF FIGURES

Fig. 1. *Sebacina deminuta* Bourd. This and all other figures have been drawn with the aid of a camera lucida, at a magnification of 3800 $\times$ , and subsequently reduced to about 0.3, i.e. to a magnification of about 1150 $\times$ . In addition, an absolute scale of dimension is included in all figures.

1, hyphal segment in fruit body; 2, young hypobasidium with the two minute nuclei before fusion; 3, 5, same with very small fusion nucleus; 4, same with fusion nucleus with two nucleoli still distinct; 6, same with both nuclei and nucleoli fused; 7, 8, hypobasidium showing enlarging fusion nucleus; 9, 10, mature hypobasidia with accompanying hyphal tip; 11, hypobasidium with prochromosomes in the nucleus; 12, same with six chromosomes in the nucleus; 13, same with six chromosomes free in the center; 14, same with transversely oriented spindle; 15, 16, same with daughter nuclei near the surface; 17, same with two daughter nuclei dividing simultaneously; 18, same with first longitudinal septum forming; 19, same with first longitudinal septum separating the two nuclei; 20, same with one nucleus dividing and two epibasidia well-developed; 21, 22, same with four nuclei but only two epibasidia; 23, group of hypobasidia showing normal hypobasidium with nuclear migration at left and abortive uninucleate body at right; 24, 25, vacuolate mature spores on empty collapsing basidia; 26, 28, 29, hypobasidia showing formation of the long epibasidia found in old fruit-bodies; 27, 30-32, epibasidia with stages in the formation of the spores; 33, spore receiving nucleus attenuated in passing through sterigma; 34, binucleate gloeocystidium with slender vacuolate tip; 35, 38, early stages in formation of gloeocystidia; 36, 37, 39, gloeocystidia showing the two nuclei fused; 40, swollen gloeocystidium with thin protoplasm and nucleus dividing; 41, mature basidium with nuclei about to enter the spores; 42, same with form characteristic of young fruit-bodies; 43, spore showing lateral germ tube at tip of which a secondary spore is forming; 44, mature spore, showing vacuolate content; 45, same with lateral germ tube ending in slender sterigma; 46-48, stages of development of uninucleate body shown in fig. 23; 49, tip of dendrophysis; 50, large erect binucleate sterile hair projecting above surface of old fruit-body; 51, spore forming from uninucleate body like that shown in fig. 23; 52, mature gloeocystidia surrounding mature hypobasidium; 53, three mature gloeocystidia showing traces of protoplasm in fine bands; 54, base of a gloeocystidium showing but one nucleus; 55, hypobasidium developing abnormally by two mycelial tubes, each of which receives one of the two non-fusing basidial nuclei.

Fig. 2. *Sebacina fugacissima* Bourd. & Galz. 1, 2, hypobasidial initials, before fusion of nuclei; 3-7, hypobasidia, fusion nucleus enlarging; 8-10, same, containing mature fusion nucleus; 11, hypobasidium, prochromosomes contracting; 12, 13, 16, hypobasidia, prochromosomes nearly fully contracted, nuclear membrane gone; 14, 15, 17, same, showing six chromosomes; 18-20, same, each containing two nuclei; 21, hypobasidium, with basal and first longitudinal septa complete; 22, same, with basal and first longitudinal septa forming; 23, same, with two daughter nuclei dividing simultaneously; 24, same, with one daughter nucleus divided, other dividing; 25, same, containing four daughter nuclei; 26, same, with four coarse epibasidia forming apically; 27, same, with four coarse epibasidia fully formed

and with a delayed nuclear division in the hypobasidium; 28-30, early stages in migration of nuclei from hypobasidia; 31-33, same, in formation of spores at tips of epibasidia; 34, 35, two basidia showing irregularities in time of formation of spores; 36, 37, basidia showing passage of nucleus through slender sterigma; 38, 39, epibasidia becoming vacuolate subsequent to migration of nuclei; 40, 41, branched hyphal tips occurring among basidia; 42, 45, 46, mature spores still on epibasidia; 43, mature spore germinating by short lateral tube; 44, secondary spore still attached to empty primary spore; 47, 49, mature cystidia.

Fig. 3. *Sebacina epigaea* (Berk. & Br.) Bourd. & Galz. 1, 2, hypobasidial initials, before fusion of the two nuclei; 3, hypobasidium containing fusion nucleus in which nucleoli are still distinct; 4-7, hypobasidia with enlarging fusion nuclei; 8, hypobasidium showing mature fusion nucleus; 9, same, in which contraction of prochromosomes has commenced; 10, same, from "stalk" of which a paraphysis has developed; 11, 12, 13, hypobasidia, each showing six small somewhat irregular chromosomes; 14, hypobasidium containing two daughter nuclei; 15, same, in which one daughter nucleus is dividing, while other has divided; 16, bit of hymenium, showing septate paraphysis and hypobasidium from apex of which four closely-massed coarse epibasidia are forming; 17-19, epibasidia, showing vacuolation and collapse after passage of nucleus; 20, 21, epibasidial tip, showing formation of spore at tip of short slender sterigma; 22-24, basidia showing empty condition after passage of nuclei along epibasidia; 25-28, hyphal tips of fruit-body in condition of active growth, showing many dividing nuclei, each having three chromosomes; 29, dendrophysis-like hyphal tip, in which dividing nuclei show three pairs of chromosomes splitting in anaphase; 30, spore forming at tip of unusually irregular epibasidium; 31, basidium, showing migration of one nucleus much delayed; 32, epibasidium ending in long cylindrical sterigma; 33-35, enlargement of spore after entrance of nucleus; 36, 37, 39, mature spores at tip of empty collapsing epibasidia; 38, 40, mature spores.

# THE PERFECT STAGE OF PHOMOPSIS STEWARTII ON COSMOS<sup>1</sup>

ARTHUR L. HARRISON

(WITH 8 TEXT FIGURES)

A disease of *Cosmos bipinnatus* L. to which the common name of stem blight is applied was recognized by Halsted (2) in New Jersey as early as 1894. Clinton (1) in 1903 reports its presence in Connecticut. Stewart (6) in 1910 states that the disease regularly makes its appearance in the *Cosmos* plantings at the New York State Experiment Station at Geneva. Professor H. H. Whetzel has informed the writer that it is present in his garden at Ithaca practically every year. In 1934 the writer observed that as many as 50 per cent of the plants were affected in the New York State Experiment Station plantings at Geneva.

Halsted (2, 3, 4) considered that stem blight of *Cosmos* is caused by a species of *Phlyctaena* since he apparently saw only filiform spores. Stewart (6), however, after observing these spores, which were stylospores, and pycnospores, sent the material to Peck (5) who described the fungus as *Phomopsis Stewartii* n. sp.

The material first used in the present study was supplied by Professor H. H. Whetzel from his garden in the autumn of 1932. In March of the following spring perithecia were observed on some of this material kept in a moist chamber for approximately five weeks. Since no perfect stage had been reported for *Phomopsis Stewartii*, the ascomycetous fungus was examined. It proved to be a species of *Diaporthe*. Since many species of *Diaporthe* have as their conidial stage a *Phomopsis*, the possibility that the *Diaporthe* and *Phomopsis Stewartii* were different stages of the

<sup>1</sup> Contribution from the Department of Plant Pathology, Cornell University. Part of the inoculation work was conducted at the New York State Agricultural Experiment Station at Geneva.

The writer is indebted to Professor H. H. Whetzel, Professor H. M. Fitzpatrick and Professor W. H. Burkholder for advice and criticism during the course of the investigation and for valuable corrections of the manuscript.



FIGS. 1-8. *Phomopsis Stewartii*.

same fungus was considered. Cultural work and inoculation experiments have demonstrated that such is the case.

*The disease.* Stem blight of cosmos is a disease of plants at approximately the blooming stage or of those weakened by parasites such as mildew or red spiders. The disease rarely makes its appearance until late August when the days shorten and the cosmos plants begin to bloom. The point of attack is usually at the nodes of the main stem or branches. Infection has not been observed on the leaves or roots.

The lesions first appear as dark brown areas, which rapidly enlarge until the stem is girdled. The parts above wilt and die. The lesions usually turn ashy gray and become dotted with numerous pycnidia visible to the eye.

#### ETIOLOGY

*Isolations.* Isolations of *Phomopsis Stewartii* were made by tissue and conidial plantings. Cultures of the *Diaporthe* were made from single asci obtained by placing small drops of an ascus suspension on the surface of agar plates. The drops were then examined under the microscope and those drops which contained a single ascus were marked. Later, after growth was visible, transfers were made to agar slants. Clonal lines of both the *Diaporthe* and *Phomopsis Stewartii* were obtained by cutting off hyphal tips. Isolates from all sources appeared to be identical in culture.

Although Stewart states that *Phomopsis Stewartii* (6) does poorly on potato agar, growth is rapid on potato dextrose agar. *Cosmos* and *Melilotus* plugs and sterilized wheat kernels also have proved to be favorable media.

On potato dextrose agar the mycelium covered the petri dish in from 3 to 5 days when mycelium was planted, but in from 7 to 10 days when a single ascus or conidium was planted in the petri dish. Abundant whitish cottony aerial mycelium developed. Certain isolates produced a darkening of the agar.

Some isolates produced pycnidia abundantly on all media while others fruited very sparingly. Perithecia were produced in culture from only one series of isolations. These were on sterilized cosmos stems planted with single ascus cultures. Several other

single ascus cultures in the same series and in other series failed to develop perithecia. Likewise, all attempts failed to produce perithecia in culture from clonal isolates of ascospore and conidial mycelium, from non-clonal conidial mycelium, and from tissue plantings.

The fungus was reisolated from the inoculated plants. It appeared to be identical with the original culture used in making the inoculations.

*Pathogenicity.* The pathogenicity of the ascigerous and conidial stages was demonstrated in the greenhouse by inoculating cosmos plants with mycelium from tissue plantings, from both clonal and non-clonal lines of ascospore and of pycnospore cultures, and with ascospores and pycnospores from portions of cosmos stems in moist chambers. The mycelium was applied to the wounded surface of the cosmos stems at the nodes, and then covered with moist cotton and protected by lead foil about the stem. Checks with agar were used in most of the experiments. The ascospores and the pycnospores were applied in water suspension with an atomizer. The plants were then placed in a moist chamber for about 48 hours.

Infection resulted in 90 per cent (61 out of 68) of the cases on plants inoculated in bloom or in a weakened condition, while in only 10 per cent (5 out of 46) on young plants. Each type of inoculum produced about the same percentage of infection. Infection did not occur in any of the 40 check plants. Old or weakened plants, in all cases, developed symptoms of stem blight much sooner than vigorously growing plants. The period of incubation and latent infection ranged from about two days to several weeks, depending on the age, size, and vigor of the cosmos plants.

Pycnidia were in evidence, in some cases, five days after inoculation. Perithecia have been observed repeatedly on portions of the artificially infected plants after these have been placed in moist chambers. In one case they developed within five weeks from the time of inoculation. Perithecia have never been observed in the field though no extensive search has been made for them. They have been produced once on diseased cosmos stems held under moist conditions under greenhouse benches. The *Diaporthe* under investigation is clearly, therefore, the perfect stage of *Phomopsis Stewartii* since cultures from *Phomopsis Stewartii* and the *Diap-*

*porthe* appear to be identical and since inoculations with both produce the disease.

**Taxonomy.** A careful study of the literature fails to reveal any mention of a species of *Diaporthe* on *Cosmos*. Material therefore was submitted to Dr. L. E. Wehmeyer. He states (letter of October 25, 1934) that "Morphologically, this material appears to be *Diaporthe Phaseolorum*, which is very close to *D. Arctii*, but has smaller spores, ascii, and perithecia in general, and the ostioles tend to be finer and more elongate."

Under *D. Phaseolorum* Wehmeyer (7) includes several forms which have certain morphological and physiological characters in common. The *Diaporthe* on *Cosmos*, although similar in many respects to certain of the forms of *D. Phaseolorum*, differs in certain other respects. The long beaks, the narrow ascospores, and the small ascii are characteristic of *D. Phaseolorum*, while the perithecia, pycnospores and length of the ascospores approach those of *D. Arctii*. Apparently, therefore, the *Diaporthe* in question occupies an intermediate position between *D. Phaseolorum* and *D. Arctii*. The writer feels that in consideration of the indefinite limits of the closely related species of *Diaporthe* a new species is involved. The name *Diaporthe Stewartii* is applied. The following description is based on the fungus as it occurs on *Cosmos*.

#### *Diaporthe Stewartii* sp. nov.

Synonym: *Phomopsis Stewartii* Peck.

**Pycnidial stage.** Pycnidia numerous, scattered, occasionally gregarious, simple or chambered, at first subepidermal, later erumpent, small,  $\frac{1}{3}$  to  $\frac{1}{2}$  mm. broad, black, provided with an ostiolar beak, 0–1 mm. long in culture or when affected *Cosmos* stalks are kept under moist conditions, beaks frequently hairy on surface near apex. Pycnospores common only in young pycnidia, oblong to subfusiform, hyaline, unicellular.  $4.6\text{--}12.5 \times 2.3\text{--}3.0 \mu$  (average  $6.6\text{--}9.2 \times 2.3\text{--}3 \mu$ ). Sporophores slender equal to or shorter than the spore. Stylospores very common, filiform, curved, flexuous or uncinate, hyaline,  $11.7\text{--}28.4 \times 1.0\text{--}1.5 \mu$  (average  $16.7\text{--}21.7 \times 1.0\text{--}1.5 \mu$ ). Both types of spore ooze through the ostioles in large quantities under moist conditions. For the original description see Peck (5).

**Perithecial stage.** On dead stems of *Cosmos*, single, scattered, embedded below pycnidial stromata, globose to lens-shaped, black,

308–600  $\times$  252–420  $\mu$ . Stromata poorly developed. Beaks 1 to 1.5, rarely 2 mm. long, tapering to filiform, black with rounded tip, slightly hairy, apical pore circular. Ascii sessile, elongate-clavate, apex thickened, with pore, oozing at maturity in masses through the long ostiole, 25.1–42.9  $\times$  3.9–7.3  $\mu$  (average 31.7–37.6  $\times$  4.6–5.9  $\mu$ ), paraphyses evanescent. Ascospores biserrate, hyaline, ellipsoidal to fusoid, some slightly curved, bicellular, each cell biguttulate at maturity, 9.2–17.2  $\times$  1.3–3.3  $\mu$  (average 10.6–13.2  $\times$  2.0–2.5  $\mu$ ).

Produces stem and branch canker on *Cosmos bipinnatus*. Pycnidia found on recently killed parts. Perithecia on same parts after 3–6 weeks in moist chamber. Type material in herbarium of the Department of Plant Pathology at Cornell University, Ithaca, New York, No. 21993.

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#### EXPLANATION OF FIGURES

Fig. 1, a mature perithecium showing a portion of the filiform beak and numerous ascii ( $\times 80$ ) ; 2, mature and young ascii and ascospores, the mature ascii with a distinct thickening at the apex ( $\times 432$ ) ; 3, young perithecia arising in tissue below the subepidermal pycnidia ( $\times 80$ ) ; 4, pycnidium lined with stylospores ( $\times 168$ ) ; 5, 7, masses of mature ascii at the tips of the long perithecial beaks; 6, young lesion on *Cosmos* stem from artificial inoculation; 8, pycnidia on *Cosmos* stem from artificial inoculation.

# **TWIG BLIGHT OF THE AMERICAN BLADDER NUT CAUSED BY HYPOMYCES IPOMOEAE**

**W. H. DAVIS**

**(WITH 3 TEXT FIGURES)**

A twig blight of bladder nut shrubs (*Staphylea trifolia* L.) was first noted during the summer of 1929 and seemed to be ubiquitous on shrubs planted for ornamental purposes in the Connecticut Valley. As the cause of this blight was unrecorded in the available literature, an investigation was undertaken to solve the following problems:

1. What is the cause of the blight?
2. During what season and where in the host is the disease initiated?
3. If a causal organism, how does it overwinter; has it other hosts?
4. What controls can be suggested for this disease, considering the data collected regarding the life history of the organism?

## **SYMPTOMS**

The first symptom of this disease generally appeared during the last week in June, when the tips of young shoots showed infection in two or three adjacent internodes. At first, they appeared as if frozen since the dark ivy green bark changed to a velvet green, then a silvery gray and were somewhat curled. However, later in the season, the bark turned a purple drab color which was overlaid with the silvery waxy bloom of the stem. Afterwards, this bloom disappeared and the bark changed from an army brown to a silvery gray. The necrotic seasonal growth finally appeared blanched and a silvery gray. However, these color changes were not so pronounced in secondary infection of diseased stems which consisted of several seasons growth (FIG. 3A, C, D, E).

The bark shriveled in small, parallel furrows and remained attached during the greater part of the winter. In the spring, it often shredded and parted from the stem in small fibers. Then the white, solid, exposed wood could be observed. Furthermore, very little discoloration of the wood and pith was produced by the fungus.

The advance of the organism seemed to be arrested for a brief period when it reached a node, more especially during hot, dry weather. At the junction between infected and apparently healthy host tissue, a collar of purple brown or mouse gray, raised callous tissue was formed. Under the hand lens, this callous tissue seemed to be associated with the healthy bark and was separated from the necrotic bark by an apparent cracking of the dried necrotic tissue. When the fungus continued advancing down the twig, this collar remained behind so that a single twig sometimes bore several of these collars or terminal growth rings (FIG. 3C—1, D—3).

During a three-day period of warm, humid weather, spore masses or sporodochia appeared on the bark of diseased twigs (FIG. 3C—2, G). Sporodochia were a pinkish color when moist, but turned a creamy color when dried. They continued to form on recently infected areas during warm, humid weather in summer and in fall. Sporodochia also formed on diseased twigs which had overwintered and been exposed to warm, humid weather continuing for several days as on April 25, 1930, and May 18, 1932. However, conidia were more abundant on the sporodochia during the summer as on June 6, 1929, June 26, 1930, July 4, 1931, August 1, 1932 and July 15, 1933. Conidia not only formed on sporodochia, but on the surface of perithecia during the autumn and the spring as on September 14, 1932 and May 18, 1933.

The bark of old twigs sometimes changed from its brown color to an ashen gray and showed small, red, elevated flecks which indicated the position of the maturing perithecia. Later, the bark cracked and the dark, purple-red perithecia could be seen emerging through the parallel rifts. On old stems bearing secondary infection, the dead bark remained intact for several years, but only one generation of perithecia was formed in it (FIG. 3E, F).

The wood in old stems was not appreciably rotted by the or-

ganism, but appeared firm and highly resistant to stress and strain. However, the fungus lived in the bark and parenchyma of the main stem for several years before necrosis and perithecia appeared.

During a disease survey for the past two years, blighted shrubs were located in the Connecticut Valley extending from Vermont to Connecticut and west as far as Albany wherever bladder nut shrubs are cultured for ornamentation. Furthermore, two diseased shrubs were observed in an Ohio park and one in East Chicago, but none were located west of the Mississippi River.

#### PATHOLOGICAL ANATOMY

In buds, the hyphae penetrated between the scales and entered the meristem of the maturing twig. Stained, free-hand sections of this meristem showed intercellular and infracellular hyphae present. Furthermore, the fungus was cultured from the interior of buds collected during April 1932 which showed that the fungus overwintered in the buds (FIG. 1*A, B, G, H*).

In stems, germtubes not only entered the young lenticels, but sometimes penetrated the meristem of the twig tips directly. When the hyphae were within the stem, they readily penetrated pith cells and advanced rapidly through the medullary tissue along the main axis (FIG. 1). When meristem had formed wood tracheids, hyphae passed through the pits when advancing from pith to bark (FIG. 1*J, O, R, S*). In the bark, the parenchymatous tissue and the phloem were most often penetrated. However, hyphae were observed in the cambial layer and the medullary rays. When host tissue was collected in April 1932, disinfected and implanted on potato-dextrose agar, cultures of the fusarial type of the fungus were obtained from pith and bark. This showed that hyphae overwintered in infected twigs at the youngest collar and at the node above it. In the spring, hyphae often grew between the cells of the bark, reached the surface and sporulated during the first warm, wet weather (FIG. 1*E, B*). The leaves were not perceptibly parasitized and did not become infected when inoculated as did the stems.

## THE FUNGUS AND ITS CULTURE

Several substrata were employed for culturing the fungus: potato-dextrose agar, corn meal mush, oatmeal, carrot decoction, raw and cooked plant parts consisting of rice, potato tuber, carrot root, bean pods; stems of melilot, asparagus and bladder nut. The

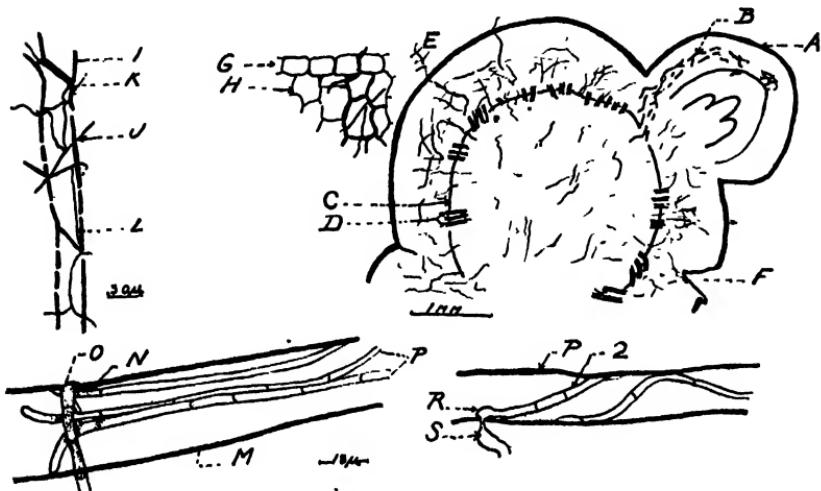


FIG. 1. *Hypomyces Ipomoeae*.

fungus grew well and sporulated on potato-dextrose agar, but perithecia formed best on melilot, asparagus and bladder nut stems. Chlamydospores were observed in water cultures and in old agar cultures. Pure cultures were obtained by isolating individuals from macroconidia, perithecia, ascii and ascospores. Here it should be stated that the microconidia failed to germinate in the cultures employed.

The acidity of the agar was determined by use of Fuller's scale because necessity demanded it. Twenty-five was the upper limit for mycelial growth; minus 32 for the lower or acid tolerance and minus 5 for maximum mycelial and conidial formation during short incubation periods. However, good mycelial growth and sporulation was observed in Petri dish cultures of agar adjusted to minus 25 F. S. and incubated at 22° C. for long periods.

The hyphae in stem tissues were 2 to 3.5 microns in diameter, averaging 3 microns; cross walls were present, but without con-

strictions, profusely branched in the phloem of the host, but without definite haustoria. In a three-day culture on potato-dextrose agar, pH 6.4 to 6.8, the mycelium was dark brown to black at the substratum, while at the surface, it was a burnt umber, van Dyke brown, or fawn color; yet, some areas showed a pinkish color (FIG. 3J, K). The growth was rather strict, seldom over 1 mm. above the surface of the agar; pinnotes and sporodochia formed on the host and in artificial cultures. These varied from a rose to a cream color, but when old and dried, they often turned a tint of brown. Yet, another monoascosporous culture showed a lemon hue which changed to rose-violet after incubating for 5 days. After 7 days, mycelium over the whole surface was purplish with a rose color at the center and a gray substratum which was somewhat pelicellate. These color changes were afterwards duplicated in another series. In general, variations in culture characters commonly associated and described for *Hypomyces* were observed in the cultures of this fungus.

Ascospores from perithecia collected in February were set to germinate in van Tieghem cells using potato-dextrose agar as a substratum. The perithecia were first washed in sterile water, then submerged in an aqueous solution of bichloride of mercury, 1:1000 for 30 seconds, washed in two changes of sterile water, a spore dilution prepared, and hanging drops placed on agar-smeared cover glasses so as to form van Tieghem cells which were then stored in a damp chamber for incubation at 22° C. After incubating for seven days, microconidia were formed and in three weeks both microconidia and macroconidia were present in a ratio of 10 to 1 (FIG. 2A, B, C, D, E, F).

Microconidia were hyaline, oblong-cylindrical, and abstricted from the ends of side branches (conidiophores) on the germtubes of germinating ascospores and other hyphae. They formed best in carrot decoction cultures of germinated ascospores which had been incubated for 24 to 48 hours at 22° C. (FIG. 2A. June 10, 1931.). Microconidia were repeatedly abstricted from the tips of conidiophores and sometimes remained in contact with each other, arranged side by side while at other times, in a somewhat globose mass or "false head" surrounded by water (FIG. 2B and C).

The sizes and shapes of microconidia varied according to the substrata employed. Two sizes were observed; the large, globose, cylindrical type and the small, often alantoid-cylindrical type. Each of these was produced from one monoascous culture which

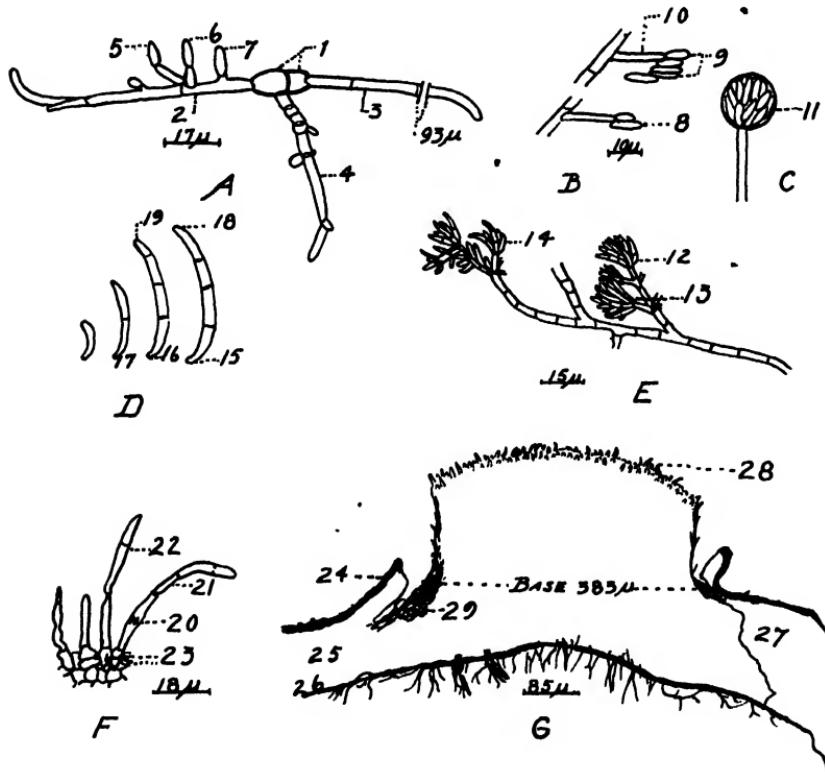


FIG. 2. *Hypomyces Ipomoeae*.

was transferred to different culture media and incubated three days. However, microconidia were cylindrical in most of the cultures.

The standard size for microconidia from the bladder nut as a host was  $2.4 \times 6.8$  microns; 50 measured.

From the above data it is to be noted that the longest microconidia were cultured in carrot decoction and from germinated ascospores; they were smallest when cultured from the stroma as in No. 4. Only one lot, No. 7, showed an average width of 3 or more microns and only one lot, No. 3, showed a length over 7

microns. The average size in culture media was  $2.3 \times 6.8$  microns as compared with  $2.8 \times 6.8$  microns when removed from the host. The limits of variation for all measurements made were  $2.4 \times 5.10$  microns (604 measured) which, considering the various substrata, compares favorably with those given by Wollenweber (8),  $3.4-4.75 \times 6-12$  microns for *Hypomyces Ipomoeae*.

TABLE 1  
MEASUREMENTS OF MICROCONIDIA COLLECTED FROM DIFFERENT  
SOURCES AND CULTURED ON POTATO-DEXTROSE  
AGAR (PDA) AND ON CARROT DECOCTION (CD)

Culture number	Culture medium	Source of inoculum	Measurements (microns)
1	PDA	Monosporous isolations	2 X 6.8
2	CD	2d transfer; monosp. culture	2.5 X 6.
3	CD	Ascospores; germtubes	2 X 10
4	CD	Pycnidiosclerotium—stroma	2 X 6
5	PDA	Host bark	2 X 7
6	PDA	Culture	2.5 X 6
7	PDA	Peritheciun attached to host	3.4 X 6.8

Macroconidia were obtained in monoascosporous cultures following the production of microconidia. They were especially prevalent on hyphae in cultures isolated from germinating ascospores and often found in sporodochia which formed on the surface of infected twigs. They also formed on perithecia, on infected young wood, on perithecioc sclerotia (stroma) located at the base of empty perithecia, and in pinnotes on agar cultures. In cultures, they formed after an incubation period varying from a minimum of two weeks to a maximum of five weeks. However, a monomacroconidial culture isolated on April 16 and incubated for 4.5 weeks contained sporodochia with both micro- and macroconidia. The macroconidia were of the fusarial type, mostly tri-septate to quinseptate. However, spores with 0, 1, 2, 3, 4, 5, 6, and 7 septa were observed. The shapes were similar to those described by Wollenweber (8); nearly cylindrical, septum zone slightly pointed, and curved ends. Base pedicellate without a distinct keel (FIG. 2D, E, F, G). The measurements in microns of fifty macroconidia in each group follow:

Group	Writer	Wollenweber, for H. Ipomoeae
3-septate .....	3.8 X 31	3.75-5 X 30-45
5-septate .....	4.0 X 56	4.25-5.5 X 45-70
7-septate .....	5 X 68	6 X 70

It is to be noted that the measurements of these macroconidia observed in culture compare favorably with those described by Wollenweber for *H. Ipomoeae* (8).

Chlamydospores were observed in agar cultures which had been prepared in November 1931 and incubated until the following March. They were scattered among plectenchymatic pellicles. This experiment was duplicated during the next year with identical results. It was also noted that when transfers were made from the chlamydosporous cultures, cultures of like type were obtained and not typical microsporous and macrosporous cultures as might be expected. Chlamydospores also formed sparingly in water cultures after incubating for one or more months. They were globose to slightly elliptical, mostly terminal and unicellular, averaging 9 microns in diameter. Wollenweber (8) describes similar chlamydospores for *H. Ipomoeae*.

Perithecia were observed on a major percentage of old infected bladder nut shrubs (FIG. 3H). From 1931 to 1933 inclusive, they made their first appearance during September, but expelled their ascospores during the following spring and summer. Perithecia were mostly gregarious; the groups averaged 0.8 mm. in length; ten of the largest groups averaged 35 perithecia each, but one group contained the maximum of 75 perithecia. Furthermore, individual perithecia were observed on bark and wood which had been exposed by wounding. The majority of the perithecia emerged through slits in the bark surrounding necrotic stems which were several years of age. Perithecia were formed on a plectenchymatic stroma in which their bases were slightly sunken (FIG. 3H). When first formed, they were a reddish-brown, but finally became much darker, often appearing a brown black. They were ovoid and their sizes were exceptionally variable as can be seen from the following table.

It is to be noted that the sizes of perithecia fluctuate and that this fluctuation is associated with the seasonal development. Wollenweber stated (8) that there is a considerable fluctuation in the average sizes of perithecia. However, measurements slightly smaller than this standard are not considered sufficient to eliminate the perithecia measured from the genus *Hyphomyces*.

TABLE 2

LIMITS OF VARIATION FOR THE MEASUREMENTS OF 50 PERITHECIA COLLECTED FROM MATERIALS UNDER DIFFERENT ENVIRONMENTS

No.	Conditions	Limits of variation (microns)	Measurements of Wollenweber
1	Spring—outdoors	126-361 X 168-420	
2	Winter—outdoors	151-235 X 150-259	
3	Herbarium—dried	128-238 X 150-272	
4	Standard—all	155-278 X 156-317	175-300 X 225-375
5	Absolute fluctuation	126-361 X 150-420	150-350 X 200-425

The exoperitheciun was verrucose, but not so abruptly as generally illustrated for *Hypomyces Ipomoeae* (FIG. 3H), but the surface of these cells often possessed the characteristic glassy luster or sheen. The endoperithecial wall consisted of minute, hyaline hyphae extending somewhat parallel to the exterior surface and some of these hyphae emerged through the ostiolum functioning as periphyses (FIG. 3H). Scattered paraphyses were formed among the asci at the base of the perithecium (FIG. 3I). Some big club-shaped cells, with large granules within, were scattered among asci and considered as abortive asci.

Asci were hyaline with a blue tinge, clavate, somewhat blunt at the apices and narrowed toward a basal cell from which they seemed to project. Limits of variation in sizes of asci follow: 5-12 X 72-94 microns; standard, 9 X 82 microns (100 measured).

Each matured, normal ascus contained eight ascospores generally arranged uniseriately (FIG. 3J). In general, these ascospores compared favorably with those described by Wollenweber for *Hypomyces Ipomoeae* except that the *exosporia* were not striated. Strict search of fresh and of herbarial materials was made, the specimens mounted in water and in lacto-phenol-green and oil immersion objectives with Zeiss lenses employed, but no striations could be detected. The measurements of ripe ascospores fluctuated only slightly (8) from those described by Wollenweber for *Hypomyces Ipomoeae* (8), 4.6-6.1 X 10-13 microns as compared with his standard of 4.5-6 X 10-13. Thus the sizes of ascospores compared favorably with Wollenweber's, but no striations were detected.



FIG. 3. *Hypomyces Ipomoeae*.

#### SYNONYMY AND CLASSIFICATION

Wollenweber (8) has presented the synonyms under which *Hypomyces Ipomoeae* has been reported. Berkley, Halsted and others reported it as a *Nectria*, but Wollenweber (7) changed it

to the genus *Hypomyces* since the ascospores are uniseptate and the polymorphic fungus bears other structures, previously mentioned, which are typical of the genus *Hypomyces*. However, contrary to Halsted's (3) findings and in accordance with Harter (4) this fungus (from the bladder nut) did not infect the sweet potato tuber. Furthermore, inoculations in fruits and stems of the eggplant failed to show infection.

Morphologically, the perithecia were of a darker color, their exterior walls less rugose, smoother, and the ascospores not striated. Otherwise, the parasite on *Staphylea* and *H. Ipomoeae* seemed sufficiently identical to be considered under one Latin binomial, but of a different forma so *Hypomyces Ipomoeae* (Hals.) Wollenw. forma *Staphyleae* is suggested for the parasite.<sup>1</sup>

#### INOCULATIONS

Living parts of the bladder nut were inoculated in the field together with excised living parts which were stored in the laboratory. The inoculum consisted of a sterile water suspension containing microconidia, macroconidia and mycelium from test tube cultures; also, germinated and ungerminated ascospores of *Hypomyces Ipomoeae* isolated from the bladder nut. The usual precautions for disinfecting surfaces—covering with glassine bags and with cellophane cylinders containing damp cotton or sphagnum moss—together with the proper preparation of checks were observed.

The laboratory inoculations were made during May, June, July and September so as to employ twigs, buds and leaves in different stages of development and under different environmental conditions. Excised twigs bearing leaves were disinfected by submerging them in an aqueous solution of mercuric bichloride for two minutes, washed in sterile water, then placed in 18-inch sterile glass jars so that the lower cut ends were buried in wet cotton. After the inoculations were made, the jars were sealed and stored at room temperature for six to twelve months. The air was suf-

<sup>1</sup> Monoascosporous and monomacroconidial cultures containing macroconidia were sent to Doctors Sherbakoff and Wollenweber. Each reported the fungus as *Hypomyces Ipomoeae*. The writer appreciates their aid in this classification.

ficiently changed during examination in an inoculating cage. This experiment was repeated in 1932 and 1933 with a total of eight separate experiments. The results showed:

1. The inoculated leaves did not become infected.
2. The wood was not perceptibly rotted.
3. The fungus produced a bark disease thereby girdling the young twigs and partially destroying the bark.
4. Microconidia, macroconidia, and perithecia formed on the diseased bark.
5. Old wood and old bark were not infected.
6. Germtubes from ascospores and macroconidia with mycelium infected young twigs so as to artificially produce symptoms identical with natural infections observed in the field.
7. Most perithecia had carbonaceous walls, were filled with oil globules and paraphysis-like hyphae, but one inoculation on May 18, 1932, produced ascospores after the culture had been incubated for six months.

Field inoculations were made during the months of April, May, June, July and September in 1931 and 1932. Bark and wood of old stems and young twigs, opening buds, closed buds, leaves, flowers and fruits were inoculated.

The best infection was obtained by an inoculation made on May 18, 1932. The inoculated young twigs showed infection in July and sporulation was noted on the surface of the bark. However, one twig did not show the symptoms until 1933, or about 14 months after inoculation. Both September inoculations failed to show infection but on June 18, 1931, an inoculation was made by placing macroconidia on opening buds, and 80 per cent infection resulted while no infection was obtained in old stems punctured and in wood inoculated. Punctured tips of young twigs showed 60 per cent infection while the unpunctured showed but 2 per cent infection. From inoculations made in the open, identical results were obtained as from those made indoors. However, infection may not become evident until the next summer following the inoculation. Furthermore, infection may cause cankers on the twigs and puncturing considerably aids infection. Opening buds and the meristem at the tip of young twigs afforded the best tissues for

entrance of the germtube. Old wood and bark were not infected. The wounds remained open, but in a good healing condition.

Data regarding the seasonal development of the disease were obtained from the inoculations and from observations in the field. In the spring time, hyphae from conidia and from ascospores penetrated the meristematic tissues in buds and in the tips of immature twigs. Infection was aided by the punctures of insects and by other wounds, but hindered by hot dry weather if not entirely prevented. A wet, warm environment was necessary for the entrance of the germtube into the meristematic tissues. About sixty days after inoculation, new conidia may appear on the infected stems and serve as a new source of inoculum.

The seasonal development as observed in the field and laboratory may be briefly stated. In the springtime, the germtubes penetrate the meristem of stems and opening buds; then, their hyphae advance downward through the young nodes and internodes until their activities are arrested by unfavorable conditions brought about by the maturing of the wood or the dry weather. Here a collar is formed in an internode and the mycelium may overwinter in the phloem and form conidia during the following spring or continue its growth during favorable conditions in late summer or autumn. Infected twig tips become necrotic and the organism advances from these tips to the older stems which may not be killed for several seasons. During the first or the second year of infection, perithecia form on the old necrotic twigs during the autumn, but only one generation of perithecia form in a given bark area.

The ascospores emerged in the spring, but did not remain viable longer than 11 months in herbarium materials and did not germinate later than August when collected from hosts in the open. Macrospores, ascospores and hyphae overwintered while attached to the host in the open and were viable in the spring of 1932. The disease was confined to the bark and the buds since leaves, floral parts, wood and fruits were not infected when inoculated. The most severe damage occurred in the young root-sprouts which sooner or later should form new stems and plants. The new stems emerged from the soil, remained in the shade under the canopy of the shrub, where conditions were generally ideal for infection

from the conidia and ascospores which fell from the infected areas above them.

#### CONTROLS

From a study of the disease and life history of the parasite, the following controls are suggested:

1. In June and July, the diseased tips of young twigs should be pruned below infected nodes and the infected materials burned at once since they bear numerous macroconidia which are potentially capable of disseminating the organism and disease.
2. In November, when the leaves have been shed, all old diseased canes should be removed and burned since perithecia form in the bark of old, diseased canes and overwinter there.
3. Prune so as to open up the shrub branches and allow air to circulate more freely around the branches and delay or often prevent infection.
4. Aphids, beetles and other insects often parasitize this shrub and disseminate the fungus. For this reason, parasitized bladder nut shrubs should be sprayed with arsenicals and with other insecticides so as to keep them free from insect vectors.
5. It seems reasonable to suppose that copper dusts and sprays applied from May to August would control the parasite since infection and spore emission take place during these months.

#### SUMMARY

1. *Hypomyces Ipomoeae* caused a twig blight of the American bladder nut.
2. The disease is initiated during the spring and summer when germinating ascospores and macroconidia penetrated meristematic tissues. Secondary infection occurs by the hyphae advancing through the phloem into the older twigs.
3. The organism overwinters as ascospores, macroconidia and hyphae within the bladder nut. No other host was observed.
4. Five controls are suggested in keeping with the life history of the fungus and seasonal development of the disease.

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## EXPLANATION OF FIGURES

Fig. 1. Tracings from camera lucida images showing the location of mycelium in stem tissues. The free-hand sections of infected bladder nut stems collected in the open were made and stained with lacto-phenol-green, washed with and mounted in clear lacto-phenol. (The drawings are partially diagrammatic.) *A*, a young infected bud in July condition; *B*, hyphae, in the primordial scale tissue; also, in the bark where it tends to form radiations from the cambium; also, from the center in the pith cells; *C*, cambium; *D*, tracheids; *E*, mycelium emerging to the surface; *F*, lenticel showing hyphae of a primary infection in the bark beneath; *G*, hyphae within bud scale tissues; *H*, meristem penetrated by hyphae; *I*, tracheids bearing hyphae, *K* and *L*; *J*, hypha threaded through a pit in the tracheal wall; *M*, an enlarged tracheid showing a hypha passing through a pit at *O*; *N*, infracellular hypha; *P*, a tracheid showing that the hypha *Q*, has formed a penetrating tube between *R* and *S*.

Fig. 2. Drawings from camera lucida images showing the formation of microconidia and macroconidia in monosporous cultures.

*A*. An ascospore incubated for 24 hours (6-10-31) on potato-dextrose agar. Tracings of a camera lucida image. 1, two cells of the germinating ascospore; 2-4, germtubes; 5-7, microconidia.

*B*. Microconidia from a culture incubated for 72 hours. 8, microconidia excised from the conidiophore tend to stick together in a group; 9, a group of microconidia from one conidiophore; 10, conidiophore.

*C*. A false conidial head. 11, group of microconidia in a drop of water.

D. Macroconidia of various sizes and different numbers of crosswalls removed from a culture incubated for 40 days on potato-dextrose agar. 15-17, tips showing a greater curve; 18-19, hilum of spores with a greater curve at the base; the 4-celled,  $3.4 \times 30.6$  and  $3.4 \times 29$ ; 2-celled,  $3.2 \times 17$ ; unicellular,  $2.4 \times 7$ .

E. Macroconidia forming on a hypha in culture; incubated for 3 weeks then dried (some conidia drawn free-hand). 12-14, macroconidia on the hyphae.

F. Macroconidia formed on conidiophores in a sporodochium from the host. Hand sectioned, stained with lacto-phenol, outlined image from a camera lucida. 20, conidiophore; 21-22, macroconidia in place; 23, foot cells and stromatic cells.

G. Diagram of a vertical, hand section through a sporodochium in the bark of the host (camera lucida employed). 24, dead bark of the host; 25, layer of infected bark; 26, cambial layer with hyphae projecting below; 27, epidermal layer, surface view; 28, tips of macroconidia crowded in the sporodochium, height, 213 microns; 29, a stroma of small, rhomboid cells formed below the basal line.

Fig. 3. Photographs showing symptoms of the twig blight and structure of the fungus, *Hypomyces Ipomoeae*, which infects the American bladder nut. A, a shrub showing numerous white tips of infected twigs. The large, diseased central leader which had been killed, was removed (3-2-'32); B, a twig infected with corynose twig blight. The unbleached condition and large, dark sclerotial areas bearing acervuli of *Coryneum* are to be contrasted with the perithecia of *Hypomyces* as shown in E and F (11-26-'31); C and D, white tips of infected twigs, at distal nodes, bearing sporodochia at C-2, and collars at 1 and 3; E, perithecia emerging through the bark of an artificially inoculated twig (11-26-'31); F, perithecia, 4, on a two-year necrotic stem; G, a sporodochium (12-12-'33), the epidermis of the host, 3; tissue with macroconidia projecting above, 6; diameter 333 microns; H, section of a stroma and nine perithecia in host tissue. Outer layers of bark, 7-8; stromatic tissue, 9; outer wall of a perithecium, 10; inner wall, 11; ascii, at the center; I, section through a perithecium highly magnified. 12, young ascus with uniseriated ascospores; 13, ascus wall and uniseptated ascospores; 14, paraphyses; J, petri dish containing a culture incubated 4 weeks. The dark areas show the locations of stromatic tissue; K, similar to J, only incubated two months and perithecia formed at 16, 17.

# DICHTOMOPHTHORA PORTULACAE, A PATHOGENE OF PORTULACA OLERACEA<sup>1</sup>

F. P. MEHRICH AND H. M. FITZPATRICK

(WITH 3 TEXT FIGURES)

The well known weed, *Portulaca oleracea* L., is perennial in Hawaii and is abundantly distributed throughout the drier pineapple growing areas on all of the islands. Here it attains a size not known on the mainland of the United States. Single plants may cover a square meter of soil and individual stems may attain a diameter exceeding 2 centimeters.

Because of the facility with which it reestablishes itself from pieces of stem and owing to the vast number of seeds produced, it is the most difficult weed to eradicate from the fields, with the exception of nut grass, *Cyperus rotundus* L.

During the course of investigations to determine the possibility of controlling the weed by artificially disseminating naturally occurring pathogens, it was learned that epiphytotes were being caused by an apparently undescribed hyphomycetous fungus.

The present paper is a description of this new organism. Another paper to be published elsewhere will discuss the epiphytology of the disease, and efforts to control purslane by its use.

## DISTRIBUTION

The organism was first isolated during 1932 from diseased purslane growing in the dry regions of the island of Maui, extending ten miles from Makawao to Pulehu. No other areas of this island were sampled.

On the island of Oahu the pathogene was first found occurring over several hundred acres in the extensive dry areas of Robinson and Kunia. It was later found at Wahiawa, Kupehau, Sanitary

<sup>1</sup> Published with the approval of the Director as Technical Paper No. 83 of the Pineapple Experiment Station of the University of Hawaii.

Flats, Kahuku, Mokapuu, Koko Head, and Honolulu. None was found at Kemoo.

It has also been found on the islands of Lanai and Molokai, but not on the island of Kauai.

#### MORPHOLOGY

The pathogene is distributed intracellularly throughout all tissues of the stem and leaves of the host, forming ramifying brown hyphae approximately  $6\ \mu$  in diameter.

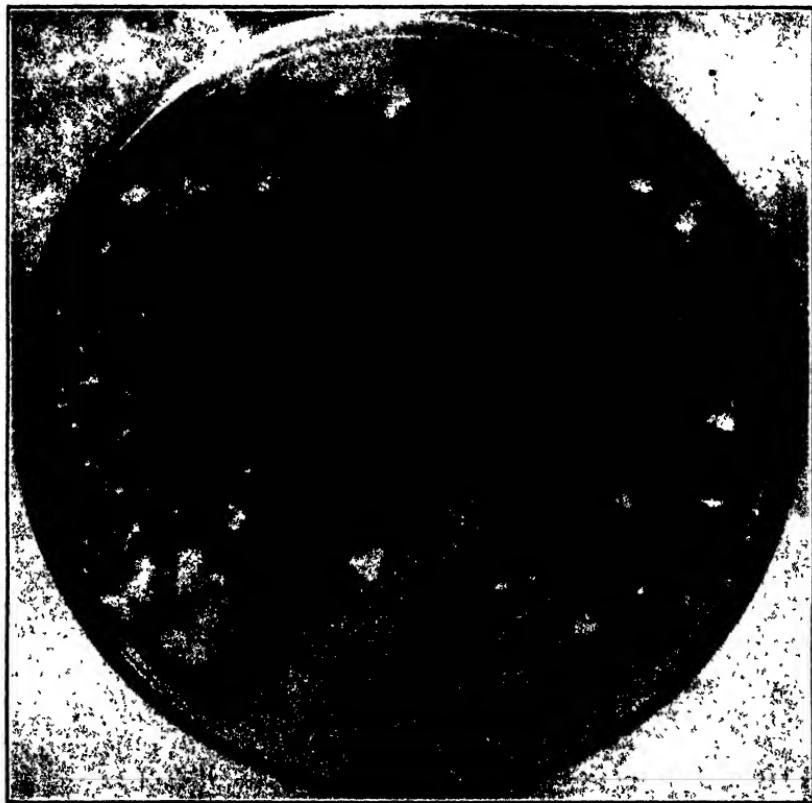


FIG. 1. A 14 day colony of A, strain L-34 and B, strain E-33 of *Dichotomophthora Portulacae* grown on malt agar.

On Difco corn meal agar, pH 6.4, the copious mycelium is at first hyaline and largely aseptate, in habit of branching resembling *Rhizoctonia* on the same medium. With age it becomes olivaceous

to black, divided into short cells, the hyphae anastomosing freely. The principal hyphae have a diameter of  $6.5\ \mu$ , while the side branches are  $5.0\ \mu$  or smaller. Neither pycnidia nor stromata are formed.

At least two distinct strains have been recognized which differ in size of conidiophores and conidia, also in character of growth on artificial media. In text figure 1 a photograph is given of a 14 day culture (A) of strain L-34 and (B) of strain E-33 on malt agar.<sup>2</sup> The rates of growth of the two are nearly the same. Strain L-34 produces conidia profusely but no sclerotia on this agar, while E-33 produces sclerotia<sup>3</sup> abundantly but conidia following desiccation only.

On *Portulaca* both produce abundant conidia, differing as described below, upon conidiophores which differ only in size.

The conidiophores are olivaceous to brown with the branching regularly dichotomous to subdichotomous in type (FIG. 2). The terminal cells of the ultimate branches are 4-8-lobed bearing a single conidium terminally on each lobe. As many as 60-75 conidia may be borne on a single conidiophore, a single branch producing as many as 14. The branches of the conidiophore occur in more than one plane in such a manner as to suggest a cyme.

As shown in text figure 2c, the conidiophores may repeatedly elongate. The lobed terminal cell while maturing spores may produce a lateral lobe which elongates, in turn producing a terminal cell, a new cluster of conidia and additional elongation, as described.

The conidiophores of strain E-33 measure  $75-100 \times 8-10\ \mu$  on malt agar. On plant material they range from  $50-220 \times 5-6.5\ \mu$ ; average approximately  $125 \times 5\ \mu$ . The conidiophores of strain L-34 are significantly longer, ranging from  $125-285 \times 4-5\ \mu$ ; average approximately  $220 \times 5\ \mu$ .

The conidia are dark brown, rarely slightly curved, ovoid to elongate-ovoid; in appearance superficially resembling the spores of *Helminthosporium*. They are one- to six-celled.

<sup>2</sup> Mehrlich, F. P. Medium for growth of pythiaceous fungi. *Phytopathology* 24: 1127-1128. 1934.

<sup>3</sup> Such structures are termed bulbils by Whetzel in that they germinate by germ tubes only, true sclerotia giving rise to fruiting structures.

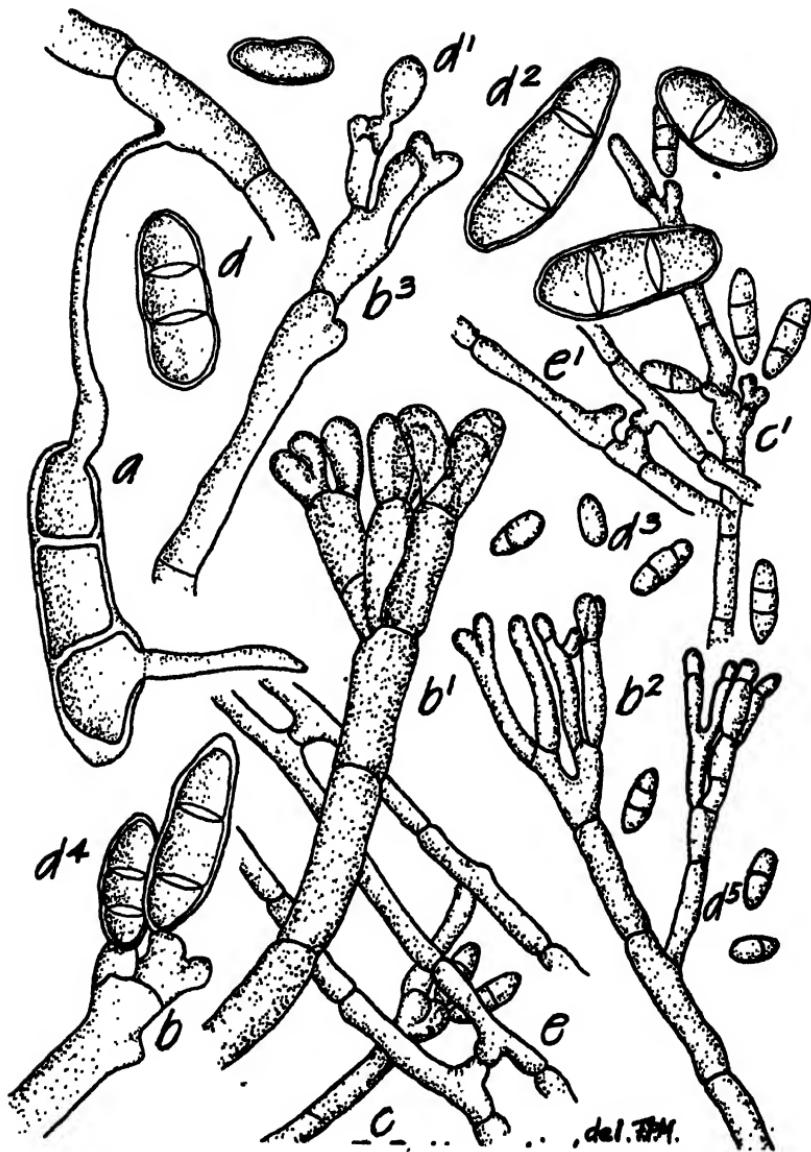


FIG. 2. The conidiophores, conidia, and anastomosing hyphae of *Di-chotomophthora Portulacae*: *a*, germinating conidium showing a germ tube fusing with the parent mycelium,  $\times 1200$ ; *b*, *b<sup>1</sup>*, *b<sup>2</sup>* ( $\times 500$ ), *b<sup>3</sup>*, portions of conidiophores showing characteristic branching and mode of conidium attachment,  $\times 1200$ ; *c*, *c<sup>1</sup>*, portions of conidiophores showing successive elongation,  $\times 500$ ; *d*, *d<sup>1</sup>*, *d<sup>2</sup>*, *d<sup>3</sup>* ( $\times 500$ ), *d<sup>4</sup>*, *d<sup>5</sup>* ( $\times 500$ ), various conidia, illustrating 1 to 3 celled types; *e*, *e<sup>1</sup>*, anastomosizing hyphae,  $\times 500$ . *det. T. M.*

TABLE 1

THE PERCENTAGE DISTRIBUTION OF CONIDIAL TYPES OF STRAINS E-33  
AND L-34 OCCURRING ON MALT AGAR AND *Portulaca oleracea*

Spore classes	Strain E-33		Strain L-34	
	Malt agar	<i>P. oleracea</i>	Malt agar	<i>P. oleracea</i>
1-celled.....	2.3	0.0	0.0	7.4
2-celled.....	5.8	2.2	24.1	37.4
3-celled.....	46.5	15.7	72.8	51.6
4-celled.....	43.0	70.4	3.1	3.7
5-celled.....	2.3	10.0	0.0	0.0
6-celled.....	0.0	1.7	0.0	0.0
Total spores in sample.....	86	230	195	190

In table 1 is presented the incidence of the several spore types in randomly selected samples from malt agar colonies and from plant lesions resulting from pure cultures of the two strains. Note that the greatest number of spores of strain E-33 are of the 3- and 4-celled types, whereas the majority of those of strain L-34 are of the 2- and 3-celled types. In strain L-34 there are almost no 4-celled spores and no 5- or 6-celled types. Similarly strain E-33 produces almost no 2-celled forms.

These differences are considered to be significant, since the values given are for pure cultures of the two strains grown simultaneously under similar conditions.

As shown by table 2, the size of the conidia of strain E-33 is much larger than of L-34.

Despite these differences, the essential morphology of the two strains is so similar that they are regarded as a single species.

The conidia germinate by one or more terminal or lateral germ tubes. In culture the germ tubes of adjacent spores often anastomose with each other, or fuse with the parent mycelium. In text figure 2 is given a camera lucida drawing illustrating various of these structures.

The sclerotia formed so abundantly on malt agar by strain E-33 are almost wholly lacking in strain L-34. They are formed in the former after 48-72 hours at 25° C., and after proportionate intervals throughout the temperature growth range of the organism. The sclerotia are extremely variable in size, ranging from 56  $\mu$ -203  $\mu$   $\times$  56  $\mu$ -142.24  $\mu$ . They are formed from a single hypha or

TABLE 2  
THE SIZE<sup>4</sup> IN MICRONS OF CONDIA OF STRAINS E-33 AND L-34 OCCURRING ON MALT AGAR AND *Portulaca oleracea*

Spore classes	Medium	Strain E-33		Strain L-34	
		Range	Average	Range	Average
2-celled	Malt agar	None	15.30-22.95 X	6.38-7.65	19.19 X 7.27
	<i>P. oleracea</i>	None	15.30-20.40 X	7.65	18.17 X 7.65
3-celled	Malt agar	20.32-40.64 X 10.16	30.78 X 10.16	20.40-38.25 X 7.65-10.20	27.92 X 8.05
	<i>P. oleracea</i>	25.50-38.25 X 7.80-10.20	31.65 X 8.90	22.95-28.05 X 6.63-10.20	24.34 X 10.04
4-celled	Malt agar	33.02-48.26 X 10.16	38.94 X 10.16	28.05-35.70 X 8.92-10.20	31.88 X 9.56
	<i>P. oleracea</i>	40.80-51.00 X 7.80-12.75	44.88 X 11.02	28.05-38.25 X 10.20-12.75	34.68 X 10.81
5-celled	<i>P. oleracea</i>	48.45-56.10 X 10.20-12.75	52.37 X 12.05	None	None
	<i>P. oleracea</i>	50.55-56.10 X 10.20-12.75	54.82 X 12.75	None	None

<sup>4</sup>The values are for 40 randomly selected spores in each spore class.

from a number of anastomosing hyphae, are irregular in shape, the surface black, the interior hyaline to dark gray. Each is composed of an aggregate of more or less symmetrical cells having

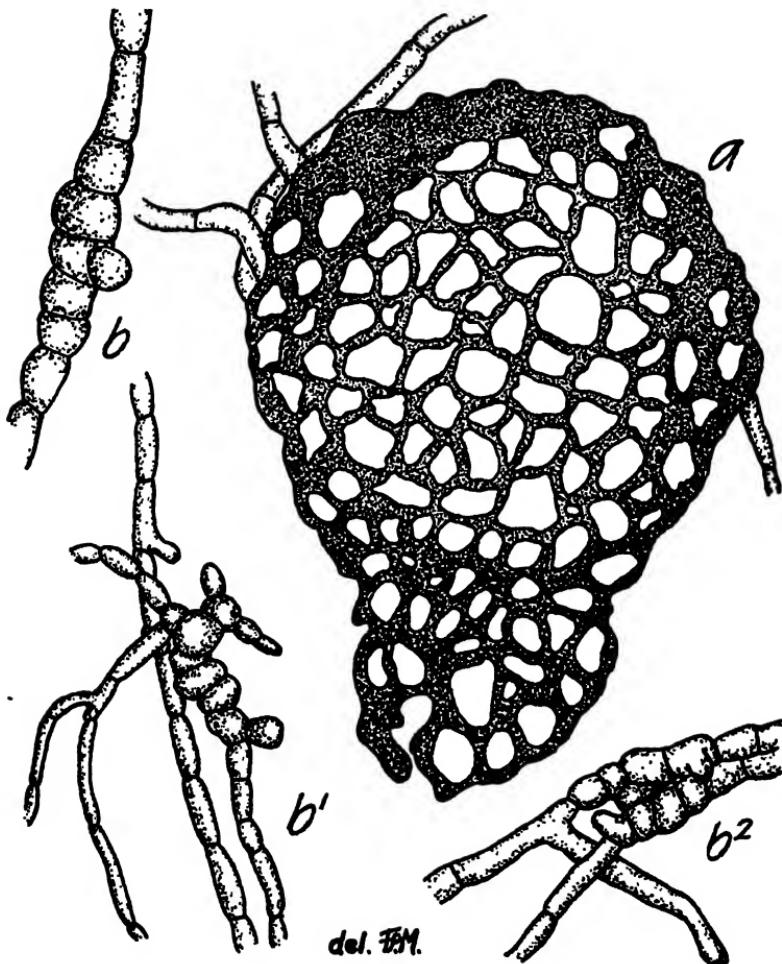


FIG. 3. Sclerotia of *Dichotomophthora Portulacae*: *a*, cross section through a mature sclerotium,  $\times 500$ ; *b*, various early stages in the formation of sclerotia, which may arise from a single hypha or by the fusion of several hyphae,  $\times 500$ .

heavy walls. They germinate by germ tubes only. In text figure 3 is shown a cross-section of a sclerotium obtained with the freezing microtome, and a few early stages in the formation of sclerotia. All drawings were made using the camera lucida.

**Dichotomophthora Portulaceae gen. & sp. nov.**

In the absence of a perfect stage, the characters detailed above place the organism in the division Macronemeae of the Phragmosporeae, but its morphology does not agree with any described genus, nor does it approach any described genus closely enough to cause confusion. A new genus is therefore established, the formal description following:

Habitat: Biogenous on *Portulaca* species

Type locality: Hawaiian Islands

Description:

Mycelium copious, at first aseptate and hyaline, soon becoming septate, olivaceous to dark brown, anastomosing freely. Pyrenidia and stromata absent. Conidiophores brown, regularly dicotomously to subdicotomously branched, successively elongating, 75–280  $\mu$  long  $\times$  5.0  $\mu$  in diameter. Terminal cells of the branches 4–8 lobed bearing a single conidium terminally upon each lobe. Conidia smooth, exogenous, one- to 6-celled, brown, ovoid to elongate-ovoid, rarely curved, 15–56  $\mu$  long  $\times$  6–13  $\mu$  wide. Sclerotia abundant, minute, black without, colorless to gray within, irregular in shape, 56–205  $\mu$  long by 56–145  $\mu$  wide.

Desiccated type-material has been deposited with the following universities: California, Chicago, Wisconsin, Cornell, and Harvard. It has also been sent to the Connecticut Agricultural Experiment Station, The New York Botanical Garden, United States Department of Agriculture, and the Imperial Institute of Mycology at Kew.

Cultures of the genus have been deposited at the Centralbureau für Schimmelpilzculturen, Baarn, Holland, and the McCormick Memorial Institute, Chicago.

## NOTES AND BRIEF ARTICLES

### MYCOLOGIA ENDOWMENT FUND

The Managing-Editor is pleased to announce the receipt of another gift which will be added to the Mycologia endowment fund held by The New York Botanical Garden. This gift was accompanied by the following note:

"I have just had some money come in, and it gives me *great pleasure* to make a (July 1) gift to Mycologia.

This is prompted in the main because of your superb contribution to the success of this magazine—and to be expended as your judgment directs."

This is the second gift of one thousand dollars made by the same person, the name of the donor being withheld by request.

It is the hope of the Managing-Editor that the interest on this endowment fund, when it has reached sufficient proportions, may be used to defray the cost of special features of the journal which cannot be carried on the regular income. The Managing-Editor takes this occasion to thank the anonymous donor for this gift.

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I have a student making a taxonomic and morphologic study of the Patellariaceae. At the present time her work is greatly hampered by a lack of fresh material. We would greatly appreciate the coöperation of the members of the Mycological Society of America in sending to us any specimens of this family they may find. Mrs. Moldenke will also be glad to identify and annotate herbarium material. Specimens should be sent to me at The New York Botanical Garden.—FRED J. SEAVER.

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### A NEW MONOGRAPH OF CORTINARIUS

Professor Jacob E. Lange has recently published a monograph in English of the Danish Cortinarii in which he keys and describes 120 species being about twenty more than half the total number of North American species described in the N. A. Flora. He accepts the six Friesian subgenera and keys them separately dividing each subgenus into two subsections. The quality of such keys is always proved by their use; however, a careful reader of these six examples will probably admit the author's claim that they are so clear and plain that a "novice may be able to follow their lead without getting side-tracked on the road." The following gen-

eralizations occur in the interesting five-page introduction: the Cortinarii always grow on the ground. None of them are really xylophilous or coprophilous; comparatively few grow in grasslands and some inhabit moss but the majority are to be found in woods preferably in deep beds of foliage or rotten needles.

The monograph is published by the Dansk Botanisk Arkiv, Copenhagen. Pr. 10 kr. The fifteen colored figures will maintain the author's high reputation for color-work in this field.  
—JOHN DEARNESS.

#### CHRONICA BOTANICA

There are nearly 4,000 Institutions of pure and applied botany. There are between 60,000 and 70,000 botanists. There are about 1,000 periodicals concerned with botany! How can you keep in touch with all this activity? How can you find out what other botanists are doing and what new work they are planning. CHRONICA BOTANICA will help you. Subscribe to it and help with the compilation of the next volume.



All directors of institutions and secretaries of societies will receive a copy of our questionnaire at the beginning of December of each year. Replies should reach the Editor-in-Chief, Dr. F. Verdoorn, Leiden, Holland, not later than January 30th, as it will generally be impossible to make use of information received after that date. Directors or Secretaries, who do not receive our preliminary circular, which will reach them annually before Oct. 15th, are kindly requested to acquaint us of the fact at their earliest convenience, which will enable us to include them in our mailing list, and will ensure their receiving a copy of the questionnaire in December.

Prospectus, sample pages and further information may be had from the EDITORIAL AND PUBLISHING OFFICE, P. O. Box 8, Leiden, Holland.





H. S. JACKSON

# MYCOLOGIA

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## THE NUCLEAR CYCLE IN HERPOBASIDIUM FILICINUM WITH A DISCUSSION OF THE SIGNIFICANCE OF HOMOTHALLISM IN BASIDIOMYCETES<sup>1, 2</sup>

H. S. JACKSON

(WITH PORTRAIT AND 4 FIGURES)

The first record of the species under discussion was made by Rostrup (1881) who distributed and described it as *Gloeosporium filicinum* Rostr. in Thümen's exsiccati, Mycotheca Universalis No. 2083. It was later independently described as *Erobasidium Brevieri* by Boudier (1900), who misinterpreted the character of the basidia. It was Lind (1908) who first recognized the true morphology of the fungus and erected the genus *Herpobasidium* with *H. filicinum* (Rostr.) as the type species. He placed the genus in the Stypinelleae of the family Auriculariaceae. Lind later (1913) added a second species, *H. Struthopteridis* (Rostr.) Lind based on *Gloeosporium Struthopteridis* Rostr. without, however, giving an adequate description. This species occurs on *Struthopteris germanica*.

<sup>1</sup> Address of the retiring President of the Mycological Society of America given at the Pittsburgh meeting.

<sup>2</sup> Contribution from the Department of Botany, University of Toronto. The writer gratefully acknowledges the assistance of Dr. S. M. Pady who prepared the slides and to Dr. Elizabeth Astrom Thompson who made the drawings for figure 3.

(MYCOLOGIA for September-October (27: 439-552) was issued  
October 1, 1935)

*Herpobasidium flicinum* (Rostr.) Lind is fairly widespread in northern Europe on *Aspidium Filix-mas* and *Aspidium Dryopteris*, and has been reported from Norway by Lind (1908) on *Aspidium Phegopteris* and *Cystopteris montana*. So far as I have been able to determine the species is unrecorded for North America. It has been found, however, in three localities in Canada. The first collection was made by the writer in a swamp south of Aurora, Ontario, in June 1932, and it has been collected there each year since that time. A second collection was made at Centreton, Ontario, June 5, 1933, by Mildred K. Nobles. It is also known from a collection made by A. E. Roland, June 25, 1933\*, near Morris-town, Kings Co., Nova Scotia. All these collections are on *Thelypteris* (*Aspidium*) *Dryopteris*.<sup>3</sup>

Lind (1908) has given a good account of the organism and a detailed description need not be repeated. Certain general features are, however, worthy of mention. When mature, that is during basidiospore production, one finds the affected fronds of *Thelypteris* showing on the upper surface uniformly scattered somewhat angular, small brownish spots. On the under surface of these discolored areas a conspicuous white mould-like growth is visible. An examination of these spots with the aid of freehand sections shows an abundant intercellular mycelium. Branches of this mycelium emerge in fascicles through the stomata and these threads continue to branch and radiate in all directions more or less parallel to the leaf surface. Such hyphae emerge from all the stomata in the discolored area, and intertwine, thus forming the white mould-like patches which form a conspicuous feature of affected fronds. There is little or no gelatinization of the cell walls of these hyphae as is the case in a considerable number of the parasitic species in this group. A careful examination has failed to reveal any connection of these external threads with the epidermal cells. They are merely branches of the intercellular mycelium which grow to the surface of the leaf through the stomata for fructification. The basidia ultimately develop from the end cells of the external mycelium, or from side branches. These basidia when mature are uniformly two celled with stout sterigmata.

\* Since the above was written a collection on the same host made by Prof. H. H. Whetzel, June 13, 1935, near White Lake, Jamesville, New York, has been received.

One of the most conspicuous characters evident in freehand sections cleared in lacto-phenol is the presence of prominent coiled haustoria (FIG. 1a). These are figured and described by Lind

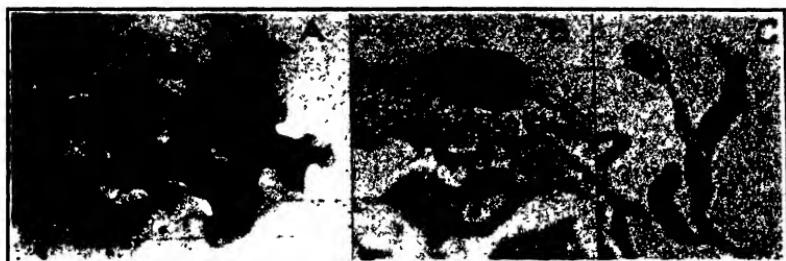


FIG. 1. *A*, haustoria in mesophyll cells; *B*, haustorium in epidermal cell (note hyphal connection to intercellular mycelium); *C*, microphotograph of basidium showing nucleus of lower cell in the spore.

(1908). The development of these has not been studied in any detail but they may occur in any cell of the mesophyll in the discolored areas and not infrequently also in the epidermal cells. As noted by Lind they are at first thin-walled and colorless, later becoming brown, due to pigment in the walls which become considerably thickened. They are of larger diameter than the intercellular or external mycelium and may become septate. Cytological examination shows that the cells of these haustoria are binucleate like the intercellular mycelium. When they occur in the epidermal cells it is evident that they have their origin from the intercellular mycelium, not from the external hyphae (FIG. 1b).

Little can be said with certainty concerning the biological history of the fungus. The evidence from field observations indicates that the fungus is systemic and perennial, presumably overwintering in the rhizomes. Infected fronds are recognizable as soon as they begin to expand in early May. There is no hypertrophy. Early in the development of the frond, the fungus seems to become localized in certain areas in the mesophyll which soon become discolored. This localization may be correlated with the development of the haustoria. There is no evidence that the fungus spreads to healthy fronds during the season. When and at what stage in the development of the host the infection occurs which results in the systemic invasion is a question on which there is no

information at present. The basidiospores are thin walled and probably short lived. It may well be that infection occurs in the buds on the rhizome or perhaps in young sporelings.

#### CYTOTOLOGICAL INVESTIGATION

Because this species developed consistently two celled basidia a cytological study of the nuclear history seemed desirable. Experience in the rusts has shown that the presence of two celled basidia is correlated in every case which has been investigated with some variation in nuclear sequence. So far as I am aware no species outside the Uredinales having this peculiarity has been examined cytologically, though several species of *Septobasidium* are known with two celled basidia.

Material for sectioning was fixed in Formalin-Acetic-Alcohol and Flemming's weaker solution. The latter fixative proved much more favorable than the former. The slides were stained with the triple stain by Dr. S. M. Pady. The primary purpose of the study was to determine the nuclear history rather than the details of nuclear division. It was considered necessary, however, to establish whether or not a fusion of nuclei took place in the young basidium, and the number and position of the divisions which followed in the developing basidium.

The cells of the intercellular mycelium, the haustoria and the external hyphae as well as of the young basidium are binucleate. No uninucleate mycelium was observed. Since, however, the initial point of infection is unknown, no young stages were examined. The two nuclei present in the young basidium fuse (FIG. 3: 1-4). No evidence of a degenerating nucleus has been observed. Figure 3: 1 shows a fusion nucleus with two nucleoli and figure 3: 2 a later stage with one large nucleolus. Figure 3: 3 shows a late prophase with four bivalent chromosomes.

Division figures of the first meiotic division of the fusion nucleus are abundant in the material studied. Figure 3: 4 shows an early metaphase in which it is evident that four chromosomes are present as well as the nucleolus. Two of these chromosomes are elongated, two appear approximately round. What appear to be central bodies are also present. Figure 3: 5 shows a section, oblique to the basidium, in which the nucleolus and one central body with the

four chromosomes are evident. Figure 3: 6 shows the division in anaphase and figure 3: 7, late telophase.

Divisions of the nuclei of the vegetative mycelium are not infrequent. Figure 3: 8 shows a late prophase with the nucleolus and four chromosomes. Figure 3: 9 illustrates a division in anaphase. Nine stained bodies are present, one of which is presumably the nucleolus. Figure 3: 10 shows a division in late telophase. Figure 3: 11 illustrates two nuclei in a haustorial cell, the lower one of which is in prophase with four chromosomes and the nucleolus, while the upper nucleus is in metaphase showing two long and two short chromosomes. Figures 3: 12 and 3: 13 are of basidiospores, the latter preparing to develop a secondary spore.

From this study it seems evident that a fusion of the two nuclei takes place in the developing basidium. The haploid chromosome number is apparently four and four chromosomes appear in the first division of the fusion nucleus of the basidium, indicating that reduction of chromosomes has taken place in this division.

A careful study of the sections failed, however, to show any conclusive evidence for the occurrence of a second division of the nuclei in the cells of the basidium. Because of the nature and size of the basidia and the fact that they occur in a more or less tangled mass, it is difficult to make observations from sectioned material. Not being entirely satisfied from a study of such material that this division was actually absent, several other methods were tried. One of these methods proved favorable and seems worthy of record. Abundant material was available of whole leaves which had been fixed in Formalin-Acetic-Alcohol. Portions of the affected fronds were placed *in toto* in lacto-phenol to which had been added separately or in mixture nigrosin, Cotton blue or acid green. When the material was allowed to stand in this mixture over night the fungus became very heavily stained. By vigorously boiling in clear lacto-phenol, the stain was gradually reduced to a point where the nuclei of the external threads and basidia stood out sharply in a clear cytoplasm. By dissecting off the mat of external hyphae and basidia and teasing this out on the slide in lacto-phenol, abundant material of detached threads and basidia for detailed examination can be made available. The method is not an exact one as it is difficult to determine the point at which the boiling should be stopped. If carried too far the material begins to disintegrate. One out of a dozen trials, how-

ever, will usually give good results. The slides are not permanent but remain clear for several days, during which time they may be studied to advantage, after which the stains gradually fade out.

By the use of this method, hundreds of entire basidia have been examined in all stages of development, and the number and position of the nuclei determined. The diagrammatic figures of figure 4 are all drawn with the aid of a camera lucida from such mounts. From a study of this material it is evident that the cells of the mycelium and the young basidia are binucleate and that the two nuclei fuse in the young basidium (FIG. 4: 16) and then divide once. During this process the basidium may elongate considerably and a septum is finally laid down between the two nuclei dividing the basidium into two approximately equal cells (FIG. 4: 15). Sterigmata then begin to develop from both cells. Commonly the sterigma of the upper cell is merely an elongation of the end of the cell (FIG. 4: 17, 18, 23, 24, 25) and that of the lower cell develops just below the septum, though this is not always the case (FIG. 4: 18, 22). The basidia show a good deal of irregularity in size and shape. Spores soon begin to develop at the tips of the sterigmata.

No evidence for a second division of the nuclei was found. The writer is convinced that no such division occurs in the basidium. The nuclei which are the products of the first division of the fusion nucleus pass into the spores without dividing. On the chance that a division might occur in the basidium and one of the resulting pair of nuclei might abort, a very careful search was made for evidence of this, but none was found.

As the spore begins to form at the end of the sterigma, it may reach a considerable size before the nucleus passes into it. Usually the cell below remains turgid for some time (FIG. 4: 24, 25), but in other cases the cell collapses gradually from below as the cytoplasm advances (FIG. 4: 20). Figure 4: 1 shows a nucleus passing through the neck of the sterigma into the spore. In this case the remaining cytoplasm is confined to the sterigma, the cell below being in a collapsed condition. A number of basidia were observed in which the nucleus had passed into the spore while the cell below and the sterigma were still filled with cytoplasm (FIG. 4: 24, 25). In such cases there is never any evidence of a degenerating nucleus

in the cell below. Figure 1c is a photomicrograph of the basidium from which figure 4: 24 was drawn.

Quite commonly an interesting irregularity was observed in the development of sterigmata and spores. These are illustrated in figure 4: 19-22. In some cases two sterigmata develop from a cell (FIG. 4: 22). In others the sterigma of one or both cells tends to branch and a spore starts to develop on each point (FIG. 4: 20, 21). In no case observed do these extra spores reach maturity. One of them receives the nucleus, the other fails to develop (FIG. 4: 20). Species in this and related groups which develop two celled basidia are quite certainly derived through simplification from forms which develop a typical four celled basidium (see discussion below). It seems possible that this abortive attempt to form more than one spore per cell is a reflection of this origin.

In mounts made in the manner described above there are many detached spores. Hundreds of these in which the nuclei are sharply stained have been examined and they are always uninucleate. These spores apparently produce secondary spores as illustrated in figure 4: 26. In no case has any evidence been obtained that the nucleus divides during the formation of the secondary spore. A careful search failed to reveal any case where the nucleus had passed into the secondary spore while it was still attached but several cases were observed of empty spores which had evidently produced a secondary one.

It would appear from this study that in *Herpobasidium filicinum* the mycelium is binucleate and that the dicaryon fuses in the young basidium. A single division follows in which the chromosome number is reduced. The nuclei so formed pass into the spores without further division. Since the mycelium is binucleate so far as observed, the question arises as to the origin of this dicaryon from the uninucleate spore. Unfortunately no observations have yet been made on this phase of the problem. Preliminary efforts to germinate the spores resulted in failure. The writer ventures the suggestion, however, that the first division of the spore nucleus on germination will be found to initiate the binucleate phase. This division represents the second meiotic division which has been delayed.

## RELATIONSHIP

The genus *Herpobasidium* is ordinarily included in the Auriculariales, Killermann (1928) includes it as a synonym of *Helcobasidium*. It seems best for the present, however, to recognize the genus as distinct. The apparent obligate parasitism and the presence of highly developed haustoria are characters which strongly suggest a close relationship with the Uredinales. Indeed the main features which would tend to exclude the genus from the rusts are the absence of teliospores and the formation of the basidia from the terminal cells of branched external hyphae rather than in compact sori. Spore forms in the rusts may form on external hyphae as in the genera *Dasyphora*, *Edythea* and *Desmella*. Tropical rusts are known in which there is no cessation of development from the spore initial to the mature basidium as in *Chrysocyclus* (= *Holwayella*).

It is perhaps unimportant whether one considers *Herpobasidium* a member of the Auriculariales or a simplified form of the Uredinales. In any case it serves to emphasize the probable close relationship between the two groups. If this relationship is a real one it is to be expected that forms will be encountered which could be placed in either group. The possibility that the rust line is a basic one from which the Auriculariales, and perhaps other Basidiomycetes as well, have been derived through simplification and, in many cases, in the loss of the parasitic habit, should not be overlooked.

## COMPARISON WITH MICROCYCLIC RUSTS

In any case the fungus, *Herpobasidium filicinum*, invites comparison with the simplified microcyclic rusts particularly since this species develops consistently a two-celled basidium and shows an aberrant nuclear history. In the Uredinales it is only in the Micro- and Endo-forms that species are known which have these peculiarities. The nuclear history in the long cycled rusts, in all the genera in which it has been studied, is remarkably uniform in its essential features.

The variations which are known in the nuclear history in the Endo-forms have been reviewed by the Moreaus (1919) and by Dodge and Gaiser (1926) and more recently for both Endo- and

Micro-forms by Jackson (1931). For the purposes of the discussion which is to follow, it seems worthwhile to summarize briefly with the aid of a condensed diagram (FIG. 2) the types of variation which are known to exist. (Literature references except recent

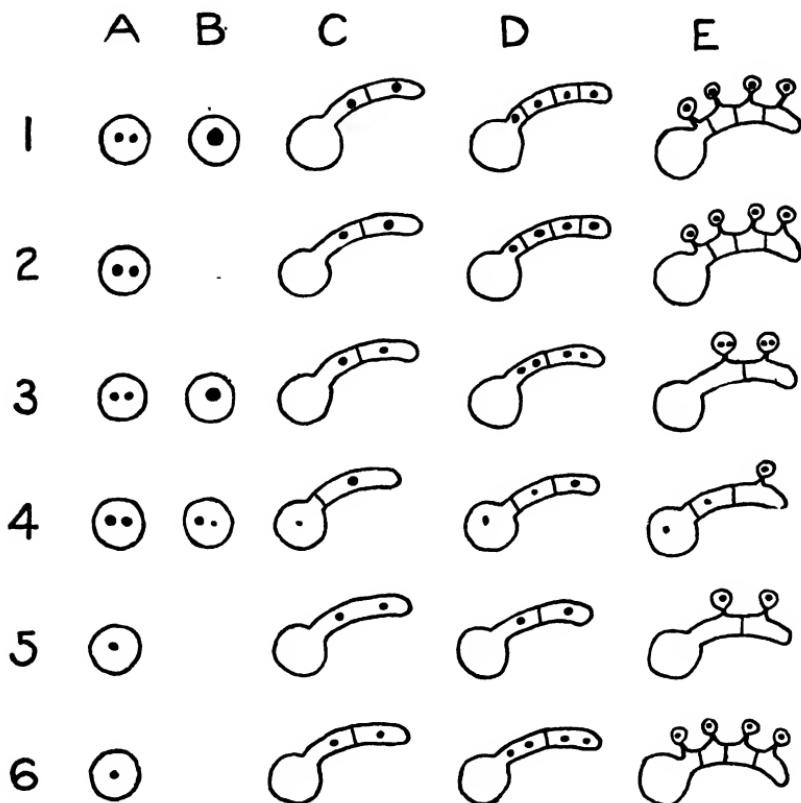


FIG. 2. Diagrams illustrating the nuclear sequence in the six variations known to occur in microcyclic rusts. A, condition in the later mycelium and young teliospore; B, fusion of nuclei where present; C, D, E, steps in the development of the basidium with nuclear condition indicated.

ones are omitted. For these the reader is referred to the papers cited above.) Six distinct variations have been described. The majority of these are known from studies of the Endo-forms. While many Micro-forms have been examined for the initiation of the binucleate phase, very few species have been studied as to the nuclear detail of the germination of the teliospores.

The six variations are as follows:

1. The mycelium is at first uninucleate, becoming binucleate in connection with the development of the sorus plexus. A fusion of the two nuclei occurs in the young teliospore or aeciospore. Meiosis follows, resulting in four nuclei and a four celled basidium and uninucleate basidiospores. Ex. *Puccinia Malvacearum* Bert., *Endophyllum Sempervivi* (A. & S.) DeBy., *Gymnoconia nitens* (Schw.) Kern & Thurst., (according to Kunkel).

2. The mycelium is uninucleate, becoming binucleate. In the young spore (morphologically the aeciospore) no fusion occurs. The two nuclei pass into the developing basidium and each divides once. Septa are laid down between each of the nuclei and the mature basidium is four celled. Four uninucleate basidiospores develop. Ex. *Gymnoconia nitens* (Schw.) Kern & Thurst. (according to Dodge), *Endophyllum Euphorbiac-sylvaticae* (DC.) Wint.

3. The mycelium is binucleate throughout. The nuclei fuse in the young teliospore. In the developing basidium a septum is laid down after the first meiotic division. No septum is formed following the second division. The mature basidium is two celled, each basidiospore receiving two nuclei, the product of the second meiotic division. Ex. *Puccinia Arenariae* (Schum.) Wint., (Lindfors, 1924), *P. Anemones-virginianae* and *P. Heucherae* (Lehmann, ined.)

4. The mycelium is at first uninucleate, becoming binucleate. The young teliospore is binucleate but no fusion occurs. One of the two nuclei degenerates. The remaining nucleus passes into the promycelium and is isolated by a cross wall. It then divides once. A cross wall is laid down between the daughter nuclei. The mature basidium is two celled but a basidiospore is formed only from the upper cell, the nucleus in the lower cell degenerating. Ex. *Endophyllum Valerianae-tuberosae* R. Maire.

5. The mycelium throughout is uninucleate as well as the young teliospore. In the basidium the single nucleus divides once and a septum is laid down between the resulting nuclei. The mature basidium is two celled, the basidiospores uninucleate. Ex. *Uromyces Rudbeckiae* Diet. & Holw., *Endophyllum Centranthi-rubri* Poir., *Gymnoconia nitens* (Schw.) Kern & Thurst.

6. The mycelium is uninucleate throughout, the nucleus of the young teliospore passes into the basidium and divides twice. Septa are laid down between the nuclei. The mature basidium is four cell and the basidiospores uninucleate. Ex. *Endophyllum uninucleatum* Moreau. Dodge (1929) has suggested that the nuclei in this form as compared with those of *E. Euphorbiae-sylvaticae* are diploid. If so, this may be correlated with the fact that a four celled basidium is formed rather than a two celled one as in other uninucleate forms.

The present study of the nuclear history of *Herpobasidium filicinum* adds another variation to this series. The mycelium, so far as observed, is binucleate. The nuclei fuse in the young basidium and the fusion nucleus divides once. A septum is laid down between the daughter nuclei and the mature basidium is two celled. The products of the first division pass into the basidiospores. The second division does not occur in the basidium and has not been observed. It seems reasonable to assume, however, that the first division of the nucleus occurring at the germination of the basidiospore represents this division and it is probable that the dicaryon initiates in this manner and that the mycelium is binucleate throughout. If this surmise is correct, then the method is comparable to that of *Puccinia Arenariae*, differing from that species in that the second division is delayed till the spore germinates.

It is of interest to speculate as to how these variations may have originated and what their significance may be.

The evidence that short cycled rusts have originated from long cycled forms through reduction is now generally accepted by most students of the group. Stated briefly the origin of the two types of microcyclic forms from the Eu-forms may occur as follows. In the Micro-forms the teliospore (the normal position of fusion of the dicaryon) appears in place of the usual first spore form—the aeciospore. In the typical Endo-forms the position of the fusion of the nuclei is moved ahead to the first spore form (the morphological aeciospore) which then germinates with a basidium (Jackson 1931).

If this explanation of the origin of microcyclic rusts from those of longer cycle is correct, then such forms are in the nature of life

cycle mutants derived through simplification from the long cycled forms. It is not necessary to assume that the long cycled form should disappear. The number of microcyclic species which may be "correlated" with the parent macrocyclic forms from which they are presumably derived is evidence that the two may continue to exist simultaneously. Since a given parent long cycled form may in all probability throw off life cycle mutants repeatedly, it follows that what is known as a species among short cycled forms may not have had any one time or place of origin. If this reasoning is sound, and assuming that the appearance of homothallism may be correlated in many forms with this process of short cycling, it does not seem surprising that variations from the nuclear history such as is typical of the parent long cycled forms, will be found in the resulting short cycled derivatives. That variations have occurred in the same "species" is indicated by the several forms of *Gymnoconia nitens* described by Dodge (1924) and Dodge and Gaiser (1926), all of which are traceable in origin to *Gymnoconia interstitialis*.

#### HOMOTHALLISM AND ITS SIGNIFICANCE

In the development of many of the short cycled forms from those of longer cycle, a change other than in life cycle has occurred, namely the appearance of homothallism. While it might be argued that insufficient facts are available to justify the statement, I venture the repetition of an opinion previously expressed (Jackson 1931) that heterothallism is a primitive character in the group. If this were not so it is difficult to account for the progressive evolution in the group and the development of the large number of species of diverse form which are now known. After all, the phenomenon which we label "heterothallism" in the fungi is essentially a provision for hybridity. It does not matter whether the "difference" implied in the term is a sexual one or is a genetic one due to factors insuring interfertility, the result is heterozygosity. The sexual process in the long cycled rusts combined with the provision for interfertility and even the greatly simplified process in the heterothallic mushrooms and related forms, is just as effective genetically as in groups having highly developed sexual structures.

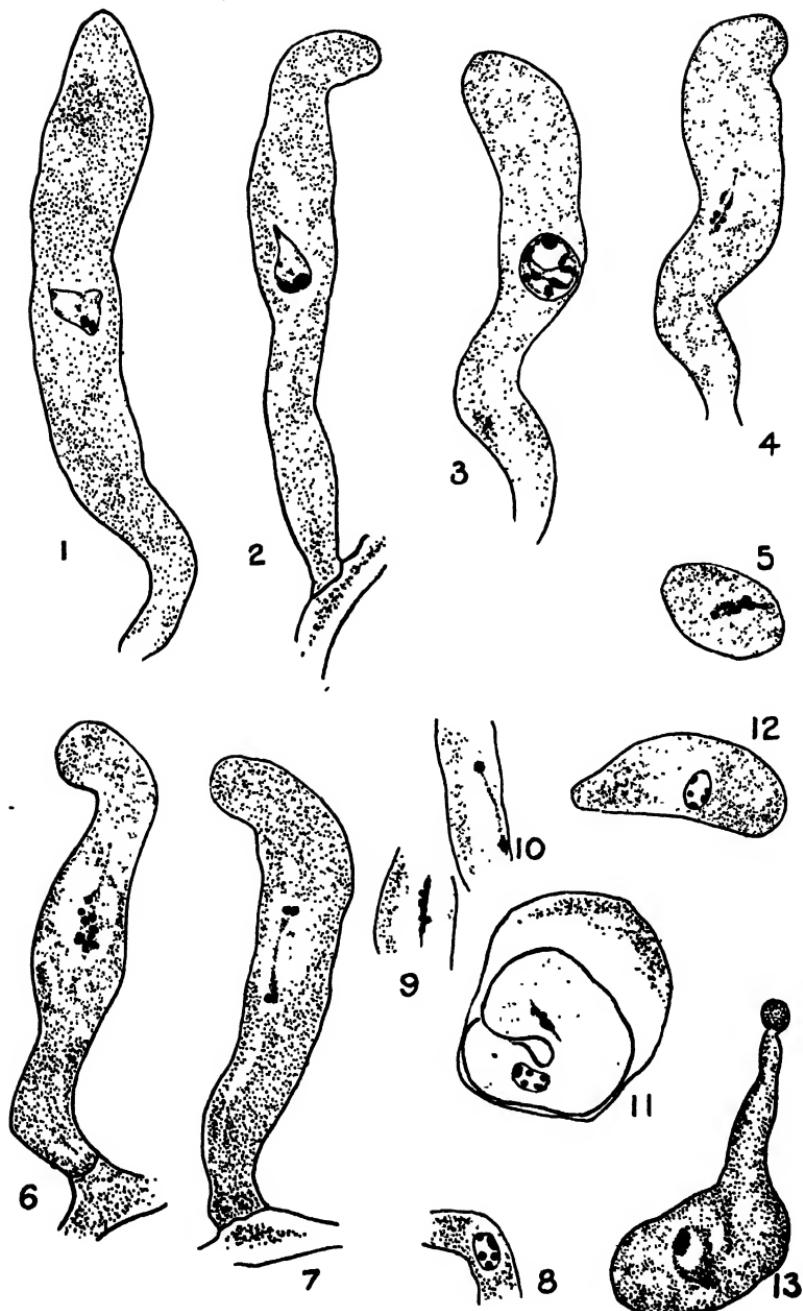


FIG. 3

The absence or loss of this provision for interfertility results in the phenomenon called homothallism.<sup>4</sup> Typically in the rusts and higher Basidiomycetes a form is homothallic when the full life cycle is carried out from a single uninucleate basidiospore. It seems probable that the great majority of microcyclic forms in the rusts will prove to be homothallic. In the case of *Puccinia Malvacearum*, Ashworth (1931) has shown experimentally that the full cycle is carried out from a single basidiospore which is at first uninucleate. From the cytological study of Allen (1933), Ashworth (1931) and earlier workers, we know the nuclear cycle in some detail. While experimental results are lacking in other short cycled forms, there is considerable evidence that homothallism is a common situation. The majority of species of Micro-*Puccinia* and *Uromyces* lack spermogonia. In many of these which have been studied cytologically, no uninucleate mycelium has been found.

Microcyclic species in which spermogonia are retained may well be either heterothallic or homothallic. Experimental work to determine the facts in individual species is greatly needed. The presence of spermogonia and spermatia, however, should not be considered indisputable evidence that the species is heterothallic. It is quite probable that in some species spermatia may be formed but are no longer functional. *Endophyllum Euphorbiae-sylvaticae* and *Gymnoconia nitens* (the form with a four celled basidium studied by Dodge and Gaiser) are known to have spermogonia, the nuclei, however, do not unite previous to the development of the basidium. Such a situation strongly suggests homothallism. It is difficult to conceive of the uninucleate forms being other than homothallic, yet *Endophyllum Centranthi-rubri* and one of the uninucleate strains of *Gymnoconia nitens* are known to develop spermogonia.

Whatever may be the situation in other forms, we know from the work of Miss Ashworth (1931) that *Puccinia Malvacearum* is

<sup>4</sup> Homothallism in the Basidiomycetes, at least in those species which complete the life cycle from a uninucleate basidiospore, should not be compared with the very special type of homothallism found in such four spored Ascomycetes as *Neurospora tetrasperma*, *Pleurage anserina* and *Gelasinospora tetrasperma* in which two nuclei of different genetic constitution are regularly included in each spore.

homothallic. In this species the basidiospore is uninucleate. This nucleus is the product of a meiotic division (Allen, 1933). Upon infection a mycelium develops which is at first uninucleate. During the development of the sorus plexus binucleate cells appear. Ashworth (1931) has made some observations on the origin of these. Whether these binucleate cells arise from nuclear migration or from a failure of the septa to form is not essential to the present discussion. The important thing to appreciate is that this does not occur just at one place but is a multiple affair. A large number of binucleate cells appear at various points in the sorus plexus initiating a number of dicaryophytic threads, each of which culminates in the formation of one or more teliospores. The nuclei which associate and which finally fuse in the teliospore cells are the products, through successive mitoses, of the original basidiospore nucleus and are hence homozygous. In such a form one may well ask what is accomplished through fusion and the meiotic process which follows other than the reduction of chromosomes? Barring gene mutations and other possible abnormalities the two sets of chromosomes which come together in the fusion nucleus are identical.

If, then, in such a form as *Puccinia Malvacearum* homothallism results in a homozygous condition and if the nuclei of the dicaryon are essentially sister nuclei, why does a dicaryon develop at all? Having developed, why the fusion in the teliospore? That such a fusion is not essential is illustrated in *Endophyllum Euphorbiae-sylvaticae* and *Gymnoconia nitens*. The nuclei having fused, it is not difficult to account for the appearance of a four celled basidium in *P. Malvacearum*, since the four celled nature of the basidium is correlated with the necessity of meiotic divisions, but why should a four celled basidium develop in *E. Euphorbiae-sylvaticae* and *Gymnoconia nitens* where the nuclei have not fused and no reduction division is necessary? In the uninucleate forms why is a two celled basidium formed in some species and a four celled basidium in others? Why is a basidium formed at all in uninucleate forms?

If one accepts the explanation of the origin of short cycled forms given above, and if this explanation is considered in connection with the genetical implications of the appearance of homothallism

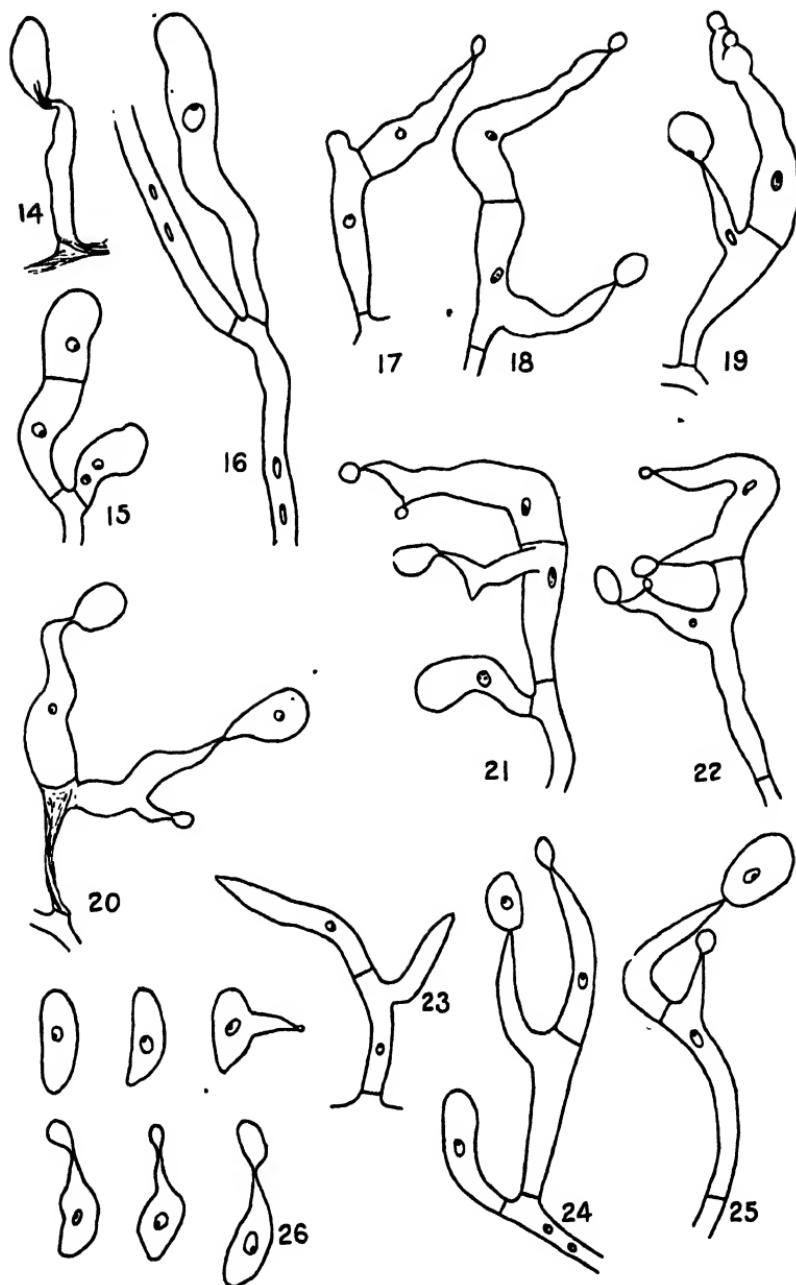


FIG. 4

in such forms, the answer to the questions raised above seems obvious. Since their origin is from long cycled heterothallic species in which a regular nuclear sequence has long become established, it is to be expected that this nuclear sequence will be retained in whole or in part in the short cycled derivatives. Should short cycled forms be developed which remained heterothallic one would expect the nuclear sequence to be the same as in the long cycled forms except that the whole process would be accomplished in one thallus. Should the appearance of homothallism be correlated with the process of short cycling then it might be expected that various modifications of the normal nuclear sequence would occur. A dicaryon might fail to develop resulting in uninucleate forms. If a dicaryon is retained it would have an entirely different origin than in heterothallic forms and the two nuclei would be genetically alike. Having formed the two nuclei might or might not fuse.

In brief, the variations which are known in the nuclear process in microcyclic rusts may be explained on the basis of phylogenetic habit, correlated with the appearance of homothallism. The normal nuclear sequence of the parent long cycled heterothallic species loses its genetic significance when the shortened cycle may be carried out through the divisions of one nucleus and for this reason variations occur.

- Where any variation of the normal process of the long cycled forms appears in the derived microcyclic forms it may be considered as indicative that the species is homothallic. The types of variations which are already known furnish an interesting and significant series in the gradual reduction of the basidium. It is quite to be expected that microcyclic forms will be discovered which no longer produce a basidium. These are to be looked for especially in the Endo-forms where the spore is a disseminating spore. In a uninucleate Endo-form the ultimate reduction would be the loss of the basidium in which case the spore would be expected to germinate with a germ tube. It is possible that the uninucleate forms of *Aecidium punctatum* Pers. and *A. leucospermum* DC. studied by Kursanov (1922) and some of the species of *Peridermium*, obviously derived from *Cronartium*, which are known to repeat themselves on *Pinus*, are of this Endo-type.

To the writer there seems no reason why a similar interpretation

may not apply to homothallic basidiomycetes in general. In *Coprinus sterquilinus* Fries, for example, the full life cycle is carried out from a single basidiospore which receives one of the four nuclei from the second meiotic division in the basidium (Sass, 1935). The spore regularly becomes binucleate before germination. On germination a multinucleate phase prevails for a time but soon a typical dicaryophytic condition develops in which there are regularly two nuclei which divide through the agency of clamp connections (Brunswik, 1924; Buller, 1931). I see no necessity to assume that because a dicaryon develops and the nuclei divide through the agency of clamp connections that this is evidence for the assumption that the two nuclei are different. They are the product of the divisions of a single nucleus. To assume a difference implies a segregation of characters at some point other than in the reduction divisions (Sass, 1929, *Text fig. 4*). Such an assumption is unnecessary and has no experimental basis. Harder's (1926) dissection experiments with this species in which a mycelium was regenerated from the temporarily uninucleate penultimate cell of a hyphal tip proves nothing in support of a difference between the two nuclei. *Coprinus sterquilinus* may be assumed to have been derived from a heterothallic species, a race of which became "self fertile." The development of a dicaryon and of clamps, the fusion in the basidium and the reduction division are all explainable on the basis of habit derived from the parental form.

The proper understanding of homothallism in all its aspects in all groups of the fungi is highly important in connection with any consideration of relationships. If heterothallism is basic and primitive and homothallism derived, then homothallic forms may be expected to show simplification, not only with reference to the nuclear history but also in morphology, particularly in the loss or modification of structures having to do with sexuality and interfertility. Such considerations are likely to prove of great importance in the development of our knowledge of relationships and interrelationships, not only in the Basidiomycetes but perhaps more particularly in the Ascomycetes.

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## EXPLANATION OF FIGS. 3 AND 4

FIG. 3. 1, fusion nucleus of basidium showing two nucleoli; 2, fusion nucleus with one large nucleolus; 3, late prophase showing four bivalent chromosomes; 4, early metaphase showing four chromosomes and nucleolus; 5,

section oblique to basidium showing nucleus in metaphase with four chromosomes and nucleolus with one central body in view; 6, a division in anaphase; 7, a division in telophase; 8, prophase of division in vegetative mycelium; 9, the same in anaphase; 10, the same in telophase; 11, the two nuclei in a haustorial cell, the lower in prophase, the upper in metaphase, showing two long and two short chromosomes; 12, basidiospore with resting nucleus; 13, same preparing to develop secondary spore. Drawn by Dr. Elizabeth Astrom Thompson.

FIG. 4. 14, nucleus passing through tip of sterigma into spore; 15, two basidia, lower one before fusion of nuclei, the other after the fusion nucleus has divided; 16, binucleate external mycelium and young basidium with fusion nucleus; 17, 18, 19, basidia forming spores, each cell uninucleate; 20, basidium in which lower cell is collapsed, the cytoplasm confined to the sterigma and the nucleus in the spore; 21, 22, basidia showing abnormal attempts to form more than one spore; in figure 22 the upper cell is developing two sterigmata; 23, basidium; 24, 25, basidia showing the nucleus of one cell in the spore; in each case the cytoplasm is clear; no evidence of abortive nuclei; 26, spores, each uninucleate, four of them showing the development of secondary spores.

# GASTERELLA, A NEW UNILOCULATE GASTEROMYCETE<sup>1</sup>

S. M. ZELLER AND LEVA B. WALKER

(WITH 13 FIGURES)

In July, 1932, a tiny, whitish, globular fungus was discovered by Dr. Abigail K. Blake on the surface of wet woodland soil in a pan protected by a glass plate sealed with vaseline. The pan of earth was being used in the greenhouse at the University of Nebraska in a series of experiments with the seed germination of prairie plants. The little fungus developed on the moisture-saturated soil along with diatoms and blue-green algae. The fructifications of this fungus for which we have chosen the generic name, *Gasterella*, were depressed globose, maturing to about 200–700  $\mu$  in diameter. They thickly dotted the surface of the soil to which they were attached below by delicate hyphae. The older mycelial hyphae were more or less gelatinous and often seemingly encrusted. As the basidiocarps matured they changed from pure white to ashy gray or even black in old age. The fruiting bodies were very abundant, new ones being constantly formed, until following a very hot day when they all died down. A new crop developed the next week when cooler weather prevailed. These matured within 3 or 4 days of their first appearance. Another hot spell killed the second crop of fructifications of the fungus, and in spite of the fact that the soil has been kept moist during the entire period since, no further growth has appeared.<sup>2</sup>

<sup>1</sup> Published as Technical Paper No. 234 with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution from the Botany departments of Oregon State College and the University of Nebraska, coöperating.

<sup>2</sup> Since the completion of this manuscript an abundance of materials of *Gasterella lutophila* has been secured on fresh loess soil from near Peru, Nebraska, which was secured through the kindness of Dr. J. M. Winter of the Peru State Teachers College. Two lots of soil were sent, the first December 12, 1934, and the second February 16, 1935. The soil in both cases was placed in containers, saturated, and closely covered. In each case an abundance of fructifications appeared in about three weeks. This seems to indicate that the fungus is very abundant in such soil.

The surface of the fructifications is dry, cottony or innately fibrillose. The peridium is about  $20\ \mu$  thick, made up of a few loosely woven hyphae mostly parallel to the surface, evidently the fundamental tissue of the earliest primordial stages.

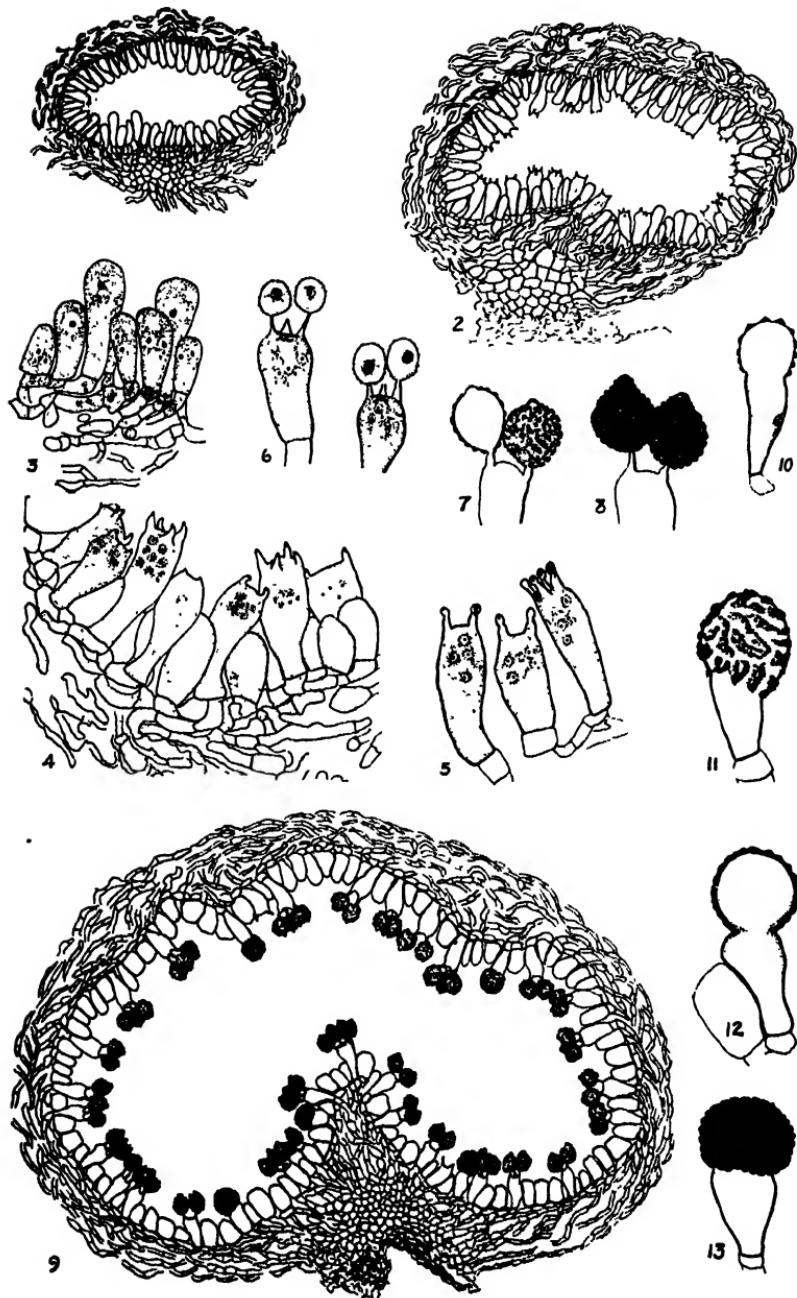
The gleba is as simple as represented in *Protogaster*,<sup>3</sup> consisting of one cavity, the wall of which is adorned by a smooth hymenial layer of clavate basidia with here and there a few capitate, clavate cystidia with dark, roughly-encrusted heads. The basidia are 2-4-spored, the latter predominant. Basidia for the most part are to be found in young fructifications. They soon collapse and disappear at maturity.

The spores are rounded below, broadly citriform with a broad apiculus. The epispore is very dark brown, quite uniformly verrucose except the apiculus which is somewhat lighter in color and almost smoothly rounded. The spores measure  $10-12 \times 12-14\ \mu$ .

Many basidiocarps representing later developmental stages were embedded and prepared slides of over 50 were studied.<sup>4</sup> Three such stages are represented by diagrammatic drawings (FIG. 1, 2, AND 9). The nuclear history in the basidium is quite the rule as found in many of the more highly developed Gasteromycetes. The cells of the subhymenial hyphae and the erect hyphal terminals in the hymenial fundaments are binucleate. In the developing holobasidium the dikaryon fuses to form a large diploid nucleus (FIG. 3). (The staining did not bring out the chromosomes.) The diploid nucleus now passes through at least two stages of division so that the young basidium beginning the growth of sterigmal protuberances is at least four nucleate. In some cases, however, in the stages of development represented by figures 4, 5, and 6, nuclei were unmistakable in the spores while four were yet to be seen in the basidium. This indicates the probable third step in division of the diploid nucleus, resulting in eight nuclei, four of which doubtless degenerate in the basidium, one migrating

<sup>3</sup> Zeller, S. M., *Protogaster*, representing a new order of the Gasteromycetes. Ann. Missouri Bot. Gard. 21: 231-249. illus. 1934.

<sup>4</sup> Materials were fixed in formal-acetic-alcohol (50 per cent alcohol 93 cc., neutral formalin 6 cc., glacial acetic acid 1 cc.) and in Bouin's fluid to which one part of urea had been added. The sectioned material was stained with Heidenhain's iron-alum-haematoxylin after which a counter stain of orange G in clove oil was added.

FIG. 1-13. *Gasterella lutophila*.

to each spore. In fact, in three cases eight very small nuclei were seen in young basidia at such a stage as represented by figure 4. Just what happens in the case of 2- and 3-spored basidia has not been observed, but all the spores seem to be uninucleate, a condition different from that in most other Gasteromycetes, except a few cases such as in *Geaster velutinus*<sup>5</sup> and *Secotium agaricoides*.<sup>6</sup> Where the dikaryon condition in the mycelium begins must for the time be left to conjecture since we have observed only binucleate, secondary mycelium.

The spores are borne symmetrically on the sterigmata as is true in other Gasteromycetes. The development of the basidia of a given basidiocarp seems to be quite irregular, so that some basidia are more advanced than others. In such cases it is impossible to distinguish primordial basidia from paraphyses. It appears, however, from the examination of mature sporocarps where all basidia have evidently matured, there still remains quite a representation of sterile cells, paraphyses.

As stated above, besides the paraphyses there are other scattering sterile cells in the hymenium, namely, cystidia. These project above the paraphyses and are up to 17  $\mu$  in diameter. One might confuse the heads of these cystidia with the basidiospores because of their color and markings, if it were not for their shape, size, and content. They are borne at the ends of basidium-like cells but are not abstricted from the basal cell, as shown in figures 10 and 12. These median sections show these spore-like heads to open into the stalk-cell and to be almost devoid of content.

The cystidia may become significant as an aid in the phylogenetic relationships of *Gasterella* and the Gasteromycetes as a class. These organs are strikingly common in many of the Thelephoraceae (*sensu lato*) on the one hand, and the Agaricaceae on the other, but they have rarely been mentioned as characteristic of the Gasteromycetes. In *Gauteria graveolens* Vitt. cystidia are conspicuous while in *G. gautieroides* (Lloyd) Zeller & Dodge and *G. morchelliformis* Vitt. they are less prominent. In these 3 spe-

<sup>5</sup> Cunningham, G. H. The development of *Geaster velutinus*. Trans. Brit. Myc. Soc. 12: 12-20. illus. 1927.

<sup>6</sup> Conrad, H. S. The structure and development of *Secotium agaricoides*. Mycologia 7: 94-104. illus. 1915.

cies of *Gauteria* the cystidia are smooth and quite thin-walled as in many of the Agaricaceae while those of *Gasterella* are more nearly like those in some genera of the Thelophoraceae and some brown-spored agarics like *Pholiota*. They are very similar in form to those of *Collybia conigena* as illustrated by Patouillard.<sup>7</sup> The cystidia of *Gasterella* seem almost like one-spored basidia which have lost their reproductive function, or if the spore-like head were fully abstracted and viable it might be interpreted as a chlamydospore.

Contrary to the condition in *Protogaster*, the place of attachment of the basidiocarp of *Gasterella* is always not only discernible but also definite. During the development of the sporocarp there is formed at the place of attachment a sterile base of pseudoparenchyma. This develops upward into the glebal cavity forming at maturity a definite conical protuberance (FIG. 9). So far as our present knowledge of the Gasteromycetes has been evolved this represents the simplest persistent form of sterile-tissue development subsequent to the fundament of the primordial sporocarp. It is therefore perhaps the most primitive of such sterile tissues yet discovered.

Because of the uniloculate gleba at maturity this genus rightfully belongs in the Protogastrales (Zeller, *l. c.*), the description of which should be amended to include forms with "simple sterile base" which is also very rarely present in *Protogaster*. *Gasterella*, however, conforms to an entirely different series of genera (so far as spore markings are concerned) than that represented by the smooth-spored genus *Protogaster*, and therefore should doubtless be referred to a new family, but we prefer now to include it in the Protogastraceae (Zeller, *l. c.*). There is no definite invagination of the locular walls in *Gasterella*.

The shape and markings of the spores in *Gasterella* place the genus definitely in the developmental line leading to *Hymenogaster*, and it is significant that certain species of *Hymenogaster* recapitulate in their early development a stage represented by the sporocarp of *Gasterella*. The morphological studies by Reh-

<sup>7</sup> Patouillard, N. Les Hyménomycètes d'Europe. 1887. (See pl. 2, f. 13.)

steiner<sup>8</sup> are convincing evidence of a direct linkage with *Hymenogaster* when viewed in the light of the development of *Gasterella*. Rehsteiner studied the development of *Hymenogaster verrucosus* Bucholtz (*H. Rehsteineri*), in the young stages of which a single locule (cavity) becomes the fundament of the gleba. In these early stages just above the place of attachment of the basidiocarp the sterile base becomes quite prominent and at these stages the development of the sporocarp of *H. verrucosus* recapitulates essentially the stage of *Gasterella* shown in figure 1. While in *Gasterella* there is no essential morphological change from this stage to maturity, in *Hymenogaster* the tramal plates emerge downward and basipetally from just beneath the upper part of the peridium as do the primordial lamellae in the single annular cavity of certain angiocarpous agarics. The same type of basipetal development in *Hymenogaster* reaches an advanced stage in species where cavities form above and continuously encroach upon the sterile tissues below until no sterile base remains at maturity. It appears therefore that *Gasterella* of the Protogastrales has its closest affinities with *Hymenogaster* through such primitive species of the genus as represented by *H. verrucosus*, and that *Hymenogaster* has a natural origin from *Gasterella* or *Gasterella*-like forms.

#### **Gasterella Zeller & Walker, gen. nov.**

Fructificationes minutae, subsphaericæ; peridium simplex primordiale contextum, indehiscens; basis sterilis pulvinata vel conica, pseudoparenchymatica; gleba uniloculata; hymenium laeve; sporae brunneæ, citriformes, apiculatae, verrucosæ.

*Fructifications* small, subspherical; *peridium* of simple fundamental tissue, indehiscent; *sterile base* pulvinate to broadly conical, pseudoparenchymatous; *gleba* unilocular; *hymenium* smooth; *spores* brown, citriform, apiculate, verrucose.

#### **Gasterella lutophila Zeller & Walker, sp. nov.**

Fructificationes oblate sphaeroideæ, 200-700  $\mu$  diametro, superficie arida, alba vel pallide brunneo-grisea, byssoidea vel innato-fibrillosa; basis sterilis pulvinata vel subconica, pseudoparenchymatica; peridium circa 20  $\mu$  crassum, simplex, ex hyphis hyalinis tenuibus laxe implicatis; gleba uniloculata, brunnea, loculus maturitate vacuus; hymenium laeve; basidia hyalina,

<sup>8</sup> Rehsteiner, H. Beiträge Zur Entwicklungsgeschichte der Fruchtkörper einiger Gasteromyceten. Bot. Zeit. 50: 764-771. pl. 10, f. 1-6. 1892.

clavata 2- vel 4-spora, sterigmatibus brevibus; cystidiis capitato-clavatis, capitulo atro-brunneo verrucoso; sporae nigro-brunneae late citriformes, apiculo lato, laevi, episporio verrucoso,  $10-12 \times 12-14 \mu$ .

*Gregaria*, ad terram uvidam in viridarium, Lincoln, Nebraska..

Type material in Walker Herbarium and Zeller Herbarium.

Fructifications depressed globose, 200-700  $\mu$  in diameter, white at first becoming light brownish drab, surface dry, cottony to innate fibrillose; sterile base pulvinate to subconical, pseudoparenchymatous; peridium of a simple layer of loosely interwoven hyphae mostly parallel with the surface, hyaline, about 20  $\mu$  thick; gleba uniloculate, brown, cavity empty; hymenium smooth; basidia clavate, 2-4-spored (mostly 4), soon evanescent; cystidia capitate-clavate, the head dark brown, verrucose like the spores; spores broadly citriform, with a broad apiculus, episporie dark brown, uniformly verrucose except the lighter colored apiculus,  $10-12 \times 12-14 \mu$ .

On the surface of damp earth in a pan, in the greenhouse. Lincoln, Nebraska. July.

#### EXPLANATION OF FIGURES

Illustrating *Gasterella lutophila* Zeller & Walker: 1, diagrammatic median section of a young fructification showing the fundamental tissue of the peridium, the pseudoparenchyma of the sterile base, and immature basidia and paraphyses,  $\times 250$ ; 2, this shows a little more advanced stage than that represented in figure 1, very immature spores developing on the sterigmata, the sterile base slightly more prominent than in figure 1,  $\times 250$ ; 3, shows the nuclear condition of the stage of development represented by figure 1,  $\times 750$ ; 4, shows a magnified section of the hymenium from the fructification illustrated in figure 2, meiosis having taken place in the nuclei,  $\times 750$ ; 5, three basidia from the same fructification as those shown in figure 4 but advanced enough to show the beginning of spore formation and the migration of the nucleus into the spore,  $\times 750$ ; 6, two basidia showing two of four immature spores; in this stage the spores are definitely uninucleate and the exospore is developing rough markings,  $\times 750$ ; 7, basidium showing two of four immature spores (one in section) with *Hymenogaster*-like markings in the exospore,  $\times 750$ ; 8, two mature, broadly rounded, broadly apiculate citriform spores showing the *Hymenogaster*-like markings,  $\times 750$ ; 9, diagrammatic section of a mature fructification (note the peg-like, conical, sterile base on a foundation of pseudoparenchyma),  $\times 250$ ; 10 and 11, immature capitate cystidia, showing the encrusted head, figure 10 a median section,  $\times 750$ ; 12 and 13, mature capitate cystidia, showing the dark roughened head markings similar to those of the spores, figure 12 a median section,  $\times 750$ .

# DIAPORTHE PHASEOLORUM ON PEPPER FRUITS<sup>1</sup>

C. M. TUCKER

(WITH 11 FIGURES)

In September, 1932, and again in 1933, mature and green fruits of pepper (*Capsicum annuum*) in a garden in Columbia, Missouri, were attacked by a fungus which causes a black, leathery type of rot extending more rapidly longitudinally than laterally in the tissue, and producing long, rather narrow lesions. In old lesions the dead tissue becomes dry and bleached (FIG. 1). Infection frequently appears to have originated at the calyx end. The lesions resemble, slightly, those resulting from infection by the anthracnose fungus, *Glomerella cingulata*, but differ in the shape and color of the infected areas and in the pliable, leathery condition of invaded tissue.

On the surface of infected areas appear minute pustulate swellings, which finally break through the epidermis. These are small, subglobose, erumpent, ostiolate fruiting bodies, the pycnidia, usually 200–300 microns in diameter, and contain two types of spores characteristic of the form genus *Phomopsis*. The condia or alpha spores are unicellular, ovoid to oblong-fusoid, hyaline, 2-guttulate,  $7.2\text{--}9.2 \times 2.2\text{--}3.2$  microns, averaging  $7.9 \times 2.7$  microns. The alpha spores develop first and germinate readily. The beta spores develop later and rarely germinate.

An examination of the literature reveals no previous record of a *Phomopsis* on pepper in America. Magnaghi (4) in 1902 described *Phoma Capsici*, on fruits in Italy, with conidia  $7\text{--}9 \times 2\text{--}3$  microns and basidia (stylospores) 20–22 microns long. These measurements are nearly identical with those of our fungus. Bianchi (2) in 1911 mentioned *Phoma Capsici* forma *caulicola*, also in Italy, as a new variety, apparently differing from *Phoma Capsici*.

<sup>1</sup> Contribution from the Department of Botany, Missouri Agricultural Experiment Station; Journal Series No. 407.

FIG. 1-6. *Diaporthe Phaseolorum.*

only in its occurrence on stems rather than fruits. In 1916 Baker (1) wrote that pepper pods dried and hung in strings became badly infected by *Phomopsis Capsici* during periods of wet weather in the Philippine Islands. The new combination, *Phomopsis Capsici*, was published by Saccardo (6) in the same year, after examination of Philippine material, although the spores in the pycnidia he examined are described as  $5-3 \times 2$  microns, considerably smaller than those described by Magnaghi. In 1919 Reinking (5) reported the same fungus again in the Philippines and Curzi and Barbaini (3) in 1927 noted its occurrence again in Italy. These records constitute our knowledge of the distribution of a *Phomopsis* on peppers.

Isolation from the infected fruit tissues yielded a white fungus which grows well on all ordinary agar media. Inoculations of detached, wounded green pepper fruits with mycelium caused a rapid rotting with the development, after 7 days, of pycnidia containing alpha type spores. After 13 days both types of spores were present. Under laboratory conditions the fungus causes a soft, wet rot which spreads over the entire fruit.

On potato dextrose agar the fungus covers the surface with white mycelium. In about 10 days black, carbonaceous pycnidia, much larger than those on pepper fruits, appear scattered singly on the agar (FIG. 2). Young pycnidia contain both conidia and stylospores, but stylospores only can be found in many older ones. In culture the spores are small, the conidia averaging  $6.1 \times 2.6$  microns and the stylospores  $14.6 \times 1.5$  microns (FIG. 3, 4).

In cultures one month old black, carbonaceous stromata, embedded in the agar, cover nearly half of the surface. Immersed in the stromata are the perithecia, usually in clusters, each 150–300 microns in diameter and tipped with a long, sinuous, irregular, carbonaceous beak bearing an ostiole (FIG. 5, 6). The asci are 8-spored, clavate,  $34.2-44.1 \times 7.2-9.0$  microns; average  $39.2 \times 7.9$  microns; the ascus wall is thin and transparent, but thickened at the apex and provided with a refringent plug pierced by a narrow pore (FIG. 11). The ascospores are obliquely uniseriate, fusoid-ellipsoid, 2-celled, constricted at the septum, guttulate, each cell containing 2 guttulae, a larger one near the septum and a smaller one near the apex,  $9-11 \times 2.5-3.5$  microns, average  $9.9 \times 3.1$  microns.

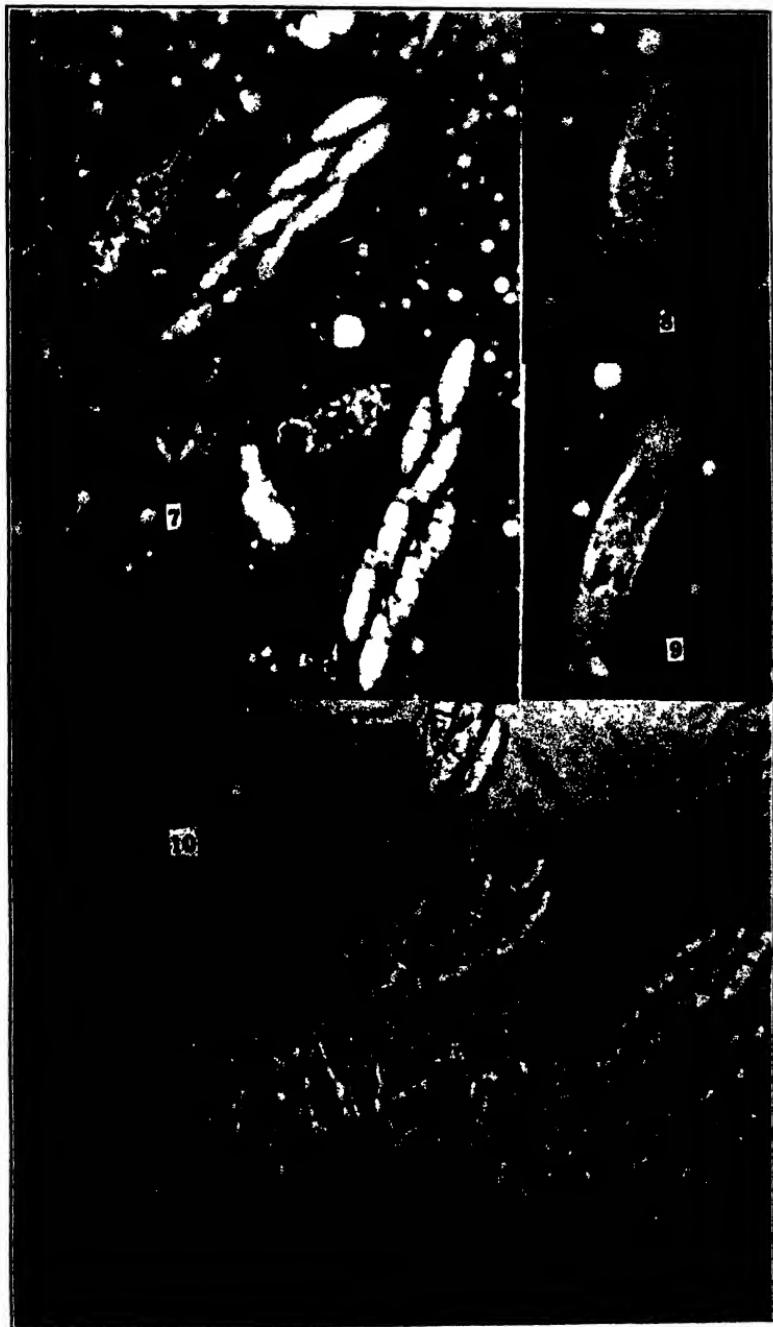


FIG. 7-11. *Diaporthe Phaseolorum*.

Seven single ascospore cultures reproduced the typical asexual and sexual stages, establishing the connection of the forms and proving the homothallic character of the mycelia from single spores, yet admitting the possibility of segregation of sex in the final nuclear division and the existence of plus and minus nuclei in the 2 cells of the spore. No single cell cultures were studied.

The sexual stage is clearly referable to the genus *Diaporthe* and was considered very similar to *D. Batatas* Hart. & Field. Wehmeyer (7) in his recent excellent monograph based on morphologic characters of the genus considers *D. Batatas* a variety of *D. Phaseolorum* (Cooke & Ellis) Sacc. and a culture of the pepper fungus sent to him was identified as a member of the group he includes in this species. *Phomopsis Capsici* (Magnaghi) Sacc. and *Phoma Capsici* forma *caulicola* Bianchi should probably be included in the list of synonyms.

The pepper isolation is of further interest as convenient material for the demonstration of nuclei in the developing ascus. When immature asci are dried on a slide in a drop of 1 per cent aqueous nigrosin solution the dye penetrates and stains the nuclei. Examination under oil without a cover glass reveals various stages from the fusion nucleus to the delineation of the spores. All stages may often be found among asci from a single perithecium (FIG. 7-10). After the third division the spore walls develop followed by the final division of the nuclei and the development of the septum. The nigrosin does not penetrate and stain the nuclei after the spore walls develop.

The plug embedded in the apex of the ascus is deeply stained. Partial destaining by adding a drop of water to the preparation brings out the plug as a ring with a small pore through the center (FIG. 11). Just how this ring functions, if it does function, as a mechanism for spore discharge, was not observed.

The fungus is maintained in living cultures in our collection and transfers are available to anyone. This easily cultured fungus and the simple, rapid method for demonstrating the nuclei and rings recommend the procedure for class room use.

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COLUMBIA, MISSOURI

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## EXPLANATION OF FIGURES

Fig. 1, lesions on fruits of *Capsicum annuum* caused by *Diaporthe Phaseolorum* (Cooke & Ellis) Sacc., natural infection; 2, pycnidia (*Phomopsis* stage) of *D. Phaseolorum* from a potato dextrose agar culture 2 weeks old ( $\times 20$ ); 3, alpha conidia produced in culture ( $\times 800$ ); 4, beta conidia produced in culture ( $\times 800$ ); 5, *D. Phaseolorum*, a single stroma bearing clustered beaked perithecia ( $\times 20$ ); 6, stromata and long beaked perithecia; from 6 weeks old potato dextrose agar culture ( $\times 20$ ); 7-10, stages of ascus development from the uninucleate stage through the third nuclear division and the initiation of ascospore delineation ( $\times 900$ ); 11, mature asci stained with nigrosin and partially destained; note the ring-like plug in the apex of each ascus ( $\times 900$ ).

## STUDIES IN THE GENUS MYCENA—II<sup>1</sup>

ALEXANDER H. SMITH

(WITH 2 FIGURES)

In this contribution sixteen species are critically discussed and four are proposed as new (*Mycena borealis*, *M. fagicola*, *M. Kauffmanii*, and *M. quinaultensis*). Most of the notes were taken from material collected in Michigan, but a collecting trip to Nova Scotia in 1931 and one to the Adirondack Mts. in New York during the season of 1934 contributed much valuable additional information.

It is the writer's purpose in this series of papers to discuss those species or groups of species within the genus which are most difficult to interpret. Only those which have some striking and unusual character are described as new. For all others the collections have been placed in the previously described species which seems to fit the writer's material most accurately.

For a critical discussion of European forms and for a generous exchange of material, the writer is particularly indebted to Mr. A. A. Pearson of England. The collecting trip to the Adirondacks was made possible through the courtesy of Assistant Dean Clyde Leavitt of the Forestry College of Syracuse University, Syracuse, N. Y. Collecting was carried on at the Archer and Anna Wild Life Forest Station near Newcomb, and at the Pack Forest near Warrensburg, N. Y. Because of favorable weather conditions at Warrensburg abundant *Mycena* flora was found, and much valuable information concerning the species described by Peck and Murrill was obtained.

### ***Mycena borealis* sp. nov.**

Caespitosa vel subcaespitosa; pileus 1–4 cm. latus, obtuse conicus demum campanulatus, umbonatus, fucus vel griseus, demum pallidus, striatus, subhygrophanus, lubricus vel subviscidus; lamellae adnatae, brevissime angulatim decurrentes, subdistantes vel distantes, venoso-reticulatae, angus-

<sup>1</sup> Papers from the Department of Botany and the Herbarium of the University of Michigan, No. 528.

tae; stipes 3–5 cm. longus, 2–4 mm. crassus, albido-fibrillosus demum glaber, fuscus vel griseus; sporae 9–10 × 4–5  $\mu$ , ellipsoidae; basidia tetraspora; cystidia 60–120 × 8–12  $\mu$ , fusoido-ventricosa demum cylindrica, apice aspera.—Specimen typicum in Herb. Mich. conservatum, prope Colchester County Nova Scotia lectum, August 27, 1931, A. H. Smith n. 759a.

Pileus 1–4 cm. broad, obtusely conic, becoming campanulate with an obtuse umbo, margin reflexed in age, colors gray with a tint of cinnamon or nearly white, "cinnamon-drab" <sup>2</sup> to "fuscous" on the umbo, fading to pale gray in age, pellucid or sulcate striate with numerous fine striations between the larger ones, subhygrophanous, lubricous to subviscid when wet, pellicle present but separable only in shreds, margin appearing slightly rimose or crenate at times, reflexed in age; lamellate adnate with a tooth, subdistant to distant, spaces between reticulate with vein-like ridges, narrow, white, no flesh tints seen in old specimens; stipe 3–5 cm. × 2–4 mm., beautifully white fibrous when young, glabrous and rather transparent in age, tough, concolorous or paler than the pileus, often compressed, white-strigose at base, gregarious to subcespitoso on wood of conifers; spores 9–10 × 4–5  $\mu$ , narrowly ellipsoid; basidia four-spored; cystidia abundant, 60–120  $\mu$ , often projecting 50–75  $\mu$ , 8–12  $\mu$  broad, fusoid-ventricose at first, cylindric in age, apex set with numerous fine projections about 2  $\mu$  long; sterile cells similar; odor none; taste slightly farinaceous.

Collections of this species from Nova Scotia where it was exceptionally abundant in 1931, from New Brunswick (among Atkinson's unnamed material), from New York by Atkinson, Mains, Smith and members of the Mycological Society of America on the 1934 summer Foray, from Michigan by Mains and Smith, and from Tennessee (alt. 6,000 ft.) by Hesler, are all constant in the characters as described. The species is almost the exact counter part of *M. polygramma* var. *albida* Kauff. in stature and consistency, but differs in the darker color, peculiar cystidia, and habitat on wood of conifers. It is also related to *M. sudora*, but the cystidia readily distinguish it.

#### *Mycena fagicola* sp. nov.

Pileus (5) 10–30 mm. latus, convexus, obtuse unbonatus vel planus, glaber, purpureo-rubrus; lamellae adnatae, confertae, angustae vel ventricosae, stramineae, acie atro-purpureae; stipes 2–4 cm. longus, 2–3 mm. crassus, dorsum rubrus, apice aurantius et fibrillosus; fibrillae atropur-

<sup>2</sup> All names of colors within quotation marks are taken from R. Ridgway, Color Standards and Color nomenclature, 1912.

pureae; succus aurantio-sanguineus, non copiosus; sporae  $7-9 \times (3) 4-5 \mu$ ; basidia tetraspora; cystidia  $30-40 \times 6-10 \mu$ , leves. Specimen typicum in Herb. Mich. conservatum, prope Cross Village Michigan lectum October 7, 1934, A. H. Smith no. 992.

Pileus (5) 10-30 mm. broad, convex, obtusely umbonate or expanded plane, at times the disk slightly depressed, pruinose at first, glabrous, disk "claret brown" when young, soon "mahogany red" to "Morocco red," in age "bay," margin "Sanford's brown" and becoming "Isabella color," sulcate striate in age, not crenate; flesh buff color, exuding an orange-yellow juice when cut; lamellae moderately close to subcrowded, bluntly adnate, narrow to moderately broad, "chamois" when young, somewhat darker in age, edge near "victoria lake" or "maroon," crenulate or even; stipe 2-4 cm.  $\times$  2-3 mm. reddish below, orange-yellow above, scantily fibrillose from "claret brown" fibrils, apex often scabrous and surface colored "claret brown" from the fibrils, purplish brown color ceasing abruptly at the apex, exuding a dull reddish brown or dull orange juice when broken or cut (juice darker in fresh young plants), base rooting somewhat among the leaves; spores  $7-9 \times 3-4 \mu$  (935)  $7-9 \times 4.5-6 \mu$  (992); basidia four-spored; cystidia on edge of gills only, or rare on sides, narrowly lanceolate and smooth,  $30-40 \times 6-10 \mu$ , content dark red. Gregarious on leaves in beech and beech-hemlock woods. Collections from Warrensburg, N. Y. (935) and Cross Village, Mich. (992) are in the Univ. of Mich. Herbarium. Collection 13941 of Atkinson is the same and is in the Atkinson Herbarium of Cornell University.

This species is perhaps most closely related to *M. cruenta*, but the yellowish gills with the dark purplish red edge and the fibrils at the apex of the stem easily separate it. *M. sanguinolenta* is a much smaller species with distant gills and a glabrous stipe which is evenly dark reddish brown in color. Atkinson in his notes commented on no. 13941 as being similar to *M. pelianthina*. There is a decided resemblance in stature, but the presence of the darkly colored juice distinguishes *M. fagicola* immediately.

#### ***Mycena Kauffmani* sp. nov.**

Pileus 1-3.5 cm. latus, conicus demum planus vel umbonatus, fuscus, demum sordidus, striatulus; lamellae confertae vel subdistantes, latae, ventricosae, uncinatae vel adnatae, albae, acie brunneae et crenulatae; stipes 1-5 cm. longus, 1-3 mm. crassus, radicatus, rigidus, pruinoso-fibrillosus; fibrillae brunneae; sporae  $6-8 \times 4-5 \mu$ , late ellipsoideae; cystidia  $35-45 \times 8-12 \mu$ , clavata vel fusoidea, apice obtusa; corticus pilei cellis pyriformibus vel

obovatus praeditus.—Specimen typicum in Herb. Univ. of Mich. conservatum, prope Dexter Mich. lectum, June 11, 1934, A. H. Smith no. 13.

Pileus 1–3.5 cm. broad, conic, becoming plane or slightly umbonate, "fuscous-black" to "fuscous" on the umbo when young, fading to "mummy brown" or "drab," margin paler, "avellanaceous" to "drab," disk slightly rugose in large specimens, surface subvelvety, dry but rather transparent, faintly striate, edge even or split at times; lamellae close to subdistant, broad, ventricose, broadly uncinate to adnexed, white, margin dull brown and minutely serrulate; stipe 1–5 cm.  $\times$  1–3 mm., equal, long rooting, at first covered by a brown pruinose-fibrous covering, scurfy at maturity, glabrous or with patches of appressed fibrils in age, tough and elastic; spores 6–8  $\times$  4–5  $\mu$ , bluntly ovate to broadly ellipsoid; basidia four-spored; cystidia on edge of gills only, 35–45  $\times$  8–12  $\mu$ , filled with a brown content, clavate to somewhat fusoid, apex obtuse; pileus with a corticated epidermis often several cells thick; odor and taste not distinctive.

In consistency, stature and structure of the pileus this species is very closely related to *M. atribrunnea* Murr., but the ellipsoid spores, lack of conspicuous cystidia on the sides of the gills and the brown gill-edge easily distinguish it. It can be readily separated from most of the species of the old section *Calodontes* by the very small spores as well as by the structure of the cap trama.

Kauffman, in The Araricaceae of Michigan, p. 792, placed this species in *M. denticulata* Peck, but later recognized that it was distinct.

#### ***Mycena quiniaultensis* Kauffm. sp. nov.**

Gregaria; pileus 1.5–2.5 cm. latus, hemisphericus demum campanulatus, umbonatus, viscidus, striatus, glaber, fuscus demum griseus, membranaceus; lamellae angustae, adnatae, brevissime angulatim decurrentes, subdistantes, albidae demum griseae; stipes 4–7 cm. longus 1.5–2.5 mm. crassus, fuscus demum griseus, glaber, viscidus; sporae 5–6  $\times$  3.5  $\mu$ , ellipsoideae; cystidia 80–110  $\times$  5–10  $\mu$ , obtuse cylindrica; basidia 30  $\times$  4–5  $\mu$ ; tetraspora.—Specimen typicum in Herb. Mich. conservatum, prope Lake Quinault, Washington lectum, October 9, 1935, C. H. Kauffman.

The following description is taken from Professor Kauffman's notes:

Pileus 1.5–2.5 cm. broad, at first subhemispheric then campanulate to subexpanded and umbonate, umbo broad, obtuse, surface viscid, striate to umbo, glabrous, at first entirely fuscous, the mar-

gin fading to "buffy brown" finally "tilleul buff," umbo fading somewhat but darker than the margin at all times; flesh thin, concolorous or paler, almost membranous; gills ascending, narrow, adnate by a decurrent tooth, subdistant at maturity, tinted gray, scarcely intervenose, edge entire and concolorous; stipe 4-7 cm.  $\times$  1.5-2.5 mm. equal, colored like the pileus, apex paler or whitish, viscid when moist, naked at apex, glabrous and even, hollow, rigid, white myceloid at base; odor and taste slight or none; spores 5-6  $\times$  3.5  $\mu$ , ellipsoid, oblong, hyaline, smooth; cystidia large, 80-110  $\times$  5-10  $\mu$ , obtuse and cylindric or slightly enlarged toward the base, smooth, arising in the gill trama; basidia 30  $\times$  4-5  $\mu$ , four-spored.

The writer collected this species in Nova Scotia (Colchester County, September 3, 1931, no. 864) on needles under fir near a small stream. The exceptionally large cystidia are unusual in the Glutinipedes. It is no doubt very closely related to *M. pelliculosa*. The gelatinous layers on both cap and stem are much thinner than in the latter species however, and the cystidia separate it readily.

**MYCENA ABRAMSII** Murr. Mycologia 8: 220. 1916.

Pileus 1-3 cm. broad, 1-2 (3) cm. high, conic campanulate or expanded and with a conic umbo, apex rather obtuse, often nearly cylindric before expanding, when young "chaetura black" fading through "fuscous" to "drab," pruinose when young and with a stanneous sheen, glabrous, hygrophanous, sulcate-striate, somewhat translucent at first, very fragile; lamellae adnate, subdistant to distant, narrow to moderately broad, pale gray, edge concolor or whitish; stipe 3-8 cm. long, 1-3 mm. thick, faintly white-fibrous near the base at first, soon concolorous with pileus or evenly pale gray with a whitish apex, covered by an easily removable bloom when young, base white strigose, very brittle; spores 10-12 (16)  $\times$  4-5.5  $\mu$ , narrowly ellipsoid to subcylindric; basidia four-spored; cystidia scattered on sides and edges of the lamellae, 40-60  $\times$  8-15  $\mu$ , smooth, fusoid-ventricose to nearly cylindric with obtuse apices; odor and taste none; gregarious to subcespitoso on leaves and debris in frondose woods.

The above description is drawn from the Michigan collections. The species has been found in the spring and fall (June and October). Murrill (10) reports it only from the type locality in California. The cystidia on the type are numerous on both the sides and edges of the lamellae, fusoid-ventricose in outline,

smooth and measure  $60-80 \times 12-16$  (20)  $\mu$ , those on the edge are usually rather short and very broad. The spores measure  $10.8-13.5 \times 5-6 \mu$  and are narrowly ellipsoid. The basidia are four-spored and measure  $32-35 \times 7-8 \mu$ .

This species is closely related to *M. aetites*, but the microscopic characters clearly separate the two. The spores are always long and narrow (measurements for different collections are as follows: No. 32-41,  $10-11 \times 4-5 \mu$ ; No. 32-522,  $12-16 \times 4-5 \mu$ ; No. 32-622,  $10-12 \times 5-5.5 \mu$ ) and the base may sometimes be drawn out into a tapering point. Whether or not this species is identical with one described earlier from Europe still remains to be determined. It may possibly be *M. consimilis* Cooke. From the writer's experience it is very difficult to distinguish *M. stannea*, *M. aetites* and *M. Abramsii* macroscopically. Microscopically they are quite readily separated. *M. stannea* has spores  $8-10 \times 5-6 \mu$ , fusoid-ventricose cystidia on the sides of the lamellae, those on the edge are provided with one or several finger like prolongations. *M. aetites* has spores practically the same size, but the cystidia are on the gill edge only and of the fusoid-ventricose type.

MYCENA AETITES Fries—*sensu* Bresadola, Iconographia 5: pl. 244.  
(FIG. 2a.)

Pileus 1.5-3 cm. broad, obtusely conic when young, becoming expanded umbonate, margin often recurved, blackish when young, "dark mouse gray" more or less after fading, evenly colored, sulcate striate on the margin, cuticle apparently splitting radially producing numerous crowded very fine striations which extend nearly to the disk, hygrophanous, moderately fragile, flesh rather thick on the disk, tapering to the margin; lamellae subdistant, adnate or toothed, narrow to moderately broad, often rather ventricose at maturity, dark gray with a paler margin; stipe 3-6 cm.  $\times$  3-4 mm. dark gray, glabrous, translucent, often compressed or twisted; rather tough; spores  $8-10 \times 5-6$  (7)  $\mu$ , ellipsoid; basidia  $27-32 \times 6-7 \mu$ , four-spored; cystidia on gill-edge only, fusoid-ventricose,  $30-35 \times 7-11 \mu$ ; odor and taste none.

The material studied was collected on needle beds in a spruce plantation late in the fall (E. B. Mains 31-675, Ann Arbor, Nov. 15, 1931). The lamellae were not as broad as indicated in Bresa-

dola's description but they were quite ventricose at maturity. In addition the spores were slightly wider than the size given by Bresadola. The fungus is apparently rather rare in the United States. For a comparison with other gray fragile species see *M. Abramsii*.

MYCENA ALCALINIFORMIS Murr. Mycologia 8: 220. 1916.

Pileus 8–15 mm. broad, conic to nearly convex, very fragile, hygrophanous, glabrous, striate to the disk "fuscous" to "hair brown" over the center, whitish gray on the margin, cinereous when faded, margin even; gills subdistant, adnate or toothed, moderately broad, gray with a whitish margin; stipe 2–4 cm.  $\times$  1–2 mm., color of cap or paler, usually whitish above, glabrous, moist and pellucid, very fragile, slightly narrowed below and mycelioid, compressed at times; odor faintly fragrant at first, soon fading; taste mild; spores  $7\text{--}9 \times 4\text{--}5 \mu$ ; basidia four-spored,  $26\text{--}29 \times 7\text{--}8 \mu$ ; cystidia on the edge only,  $26\text{--}33 \times 7\text{--}10 \mu$ , with obtuse protuberances; pileus with a pronounced vesiculose layer near the surface. Growing densely gregarious on needle beds under coniferous trees.

The spores of the type measure  $8\text{--}10.5 \times 6\text{--}7 \mu$ , no cystidia were found on the sides of the gills, those on the edge measure  $28\text{--}35 \times 5\text{--}6 \mu$  and are provided with two or more obtuse finger-like prolongations. This species has much the same stature as small forms of *M. metata* and like that species, it has a faint but fragrant odor. The colors clearly ally it to *M. ammoniaca* Fries, from which it is easily distinguished by the cystidia on the gill edge. It is paler and more fragile than either *M. alcalina* or *M. leptocephala*, two other species which also occur abundantly on needle beds and coniferous debris. The writer collected Murrill's species in great abundance on the needle beds under the older white pine stands in the Pack Forest at Warrensburg, N. Y., during the early part of September, 1934. The cystidia in all of these collections consistently possessed the obtuse finger-like prolongations at maturity, whereas those of both *M. leptocephala* and *M. ammoniaca* are smooth or rarely forked in age.

MYCENA ATRIBRUNNEA Murr. Mycologia 8: 220. 1916.

Pileus 3–15 mm. broad, obtusely conic or oval when young, becoming expanded and umbonate, umbo conic, "bister" or "snuff

brown" when fresh, fading to nearly a "buffy brown" or "fawn color," umbo darker at times but pileus often evenly colored in age, glabrous, lubricous, opaque at first, striate when fading, margin even, often splitting radially in age; lamellae narrowly adnate-uncinate to nearly free, close to subdistant, moderately broad, at times very ventricose, thick, white, pruinose under a lens, edge concolorous; stipe 1-7 cm.  $\times$  1-2 (3) mm. pure white, translucent, pruinose at the apex, glabrous, slightly rooting; spores 5-7.5  $\times$  5-6  $\mu$ , globose to subglobose, smooth; cystidia on sides and edges of the gills, abundant 60-90  $\times$  8-15  $\mu$ , ventricose to cylindric, smooth; pileus trama corticated by an area of inflated globose cells; odor and taste not distinctive.

Gregarious to scattered on rotting logs and stumps and on soil in grassy places under oak trees. In the vicinity of Ann Arbor it has nearly always been collected during the month of June. The cystidia of the type measure 80-120  $\times$  8-15  $\mu$ , are very abundant on the sides and edges of the gills, smooth, cylindric to somewhat ventricose, and with obtuse apices. The spores measure 5-6.5  $\mu$  and have a pronounced apiculus.

**MYCENA CLAVUS (Linn.) Rea, British Basidiomycetae 378.**

*Agaricus (Mycena) amabilissimus* Peck, Ann. Rep. N. Y. State Mus. 39: 39. 1887; *Mycena amabilissima* (Peck) Sacc. Syll. Fung. 9: 37. 1891; *Prunulus amabilissimus* (Peck) Murr. N. Am. Flora 9<sup>b</sup>: 324. 1916 (FIG. 1, a, b).

Pileus 3-20 mm. broad, conic, convex, campanulate or expanded-umbonate, margin at times recurved, evenly "light coral red" when young, fading to white in age or with a sordid yellowish disk; scarcely to distinctly pellucidly striate, glabrous, surface lubricous when moist; lamellae white or coral tinted, adnate, slightly ventricose but not broad, subdistant to distant, edge concolorous, intervenose; stipe 3-5 cm.  $\times$  (0.5) 1-2 mm., pure white or tinged coral red, base yellowish in age, pruinose and unpolished at first, becoming polished in age, watery fragile; spores 7-9  $\times$  3-4  $\mu$ ; basidia four-spored; cystidia present on sides and edges of lamellae; 40-65  $\times$  8-12  $\mu$ , fusoid-ventricose or with a long acuminate apex; growing singly to subcespitoso on leaves and moss.

Rea considers *M. rubella* Quél. as a synonym of *M. Clavus* and quotes von Höhnel who describes cystidia on the edge of the gills only. Boudier, whose illustration Rea cites as *M. Clavus*, clearly shows the cystidia on the sides of the gills. In all of my collections

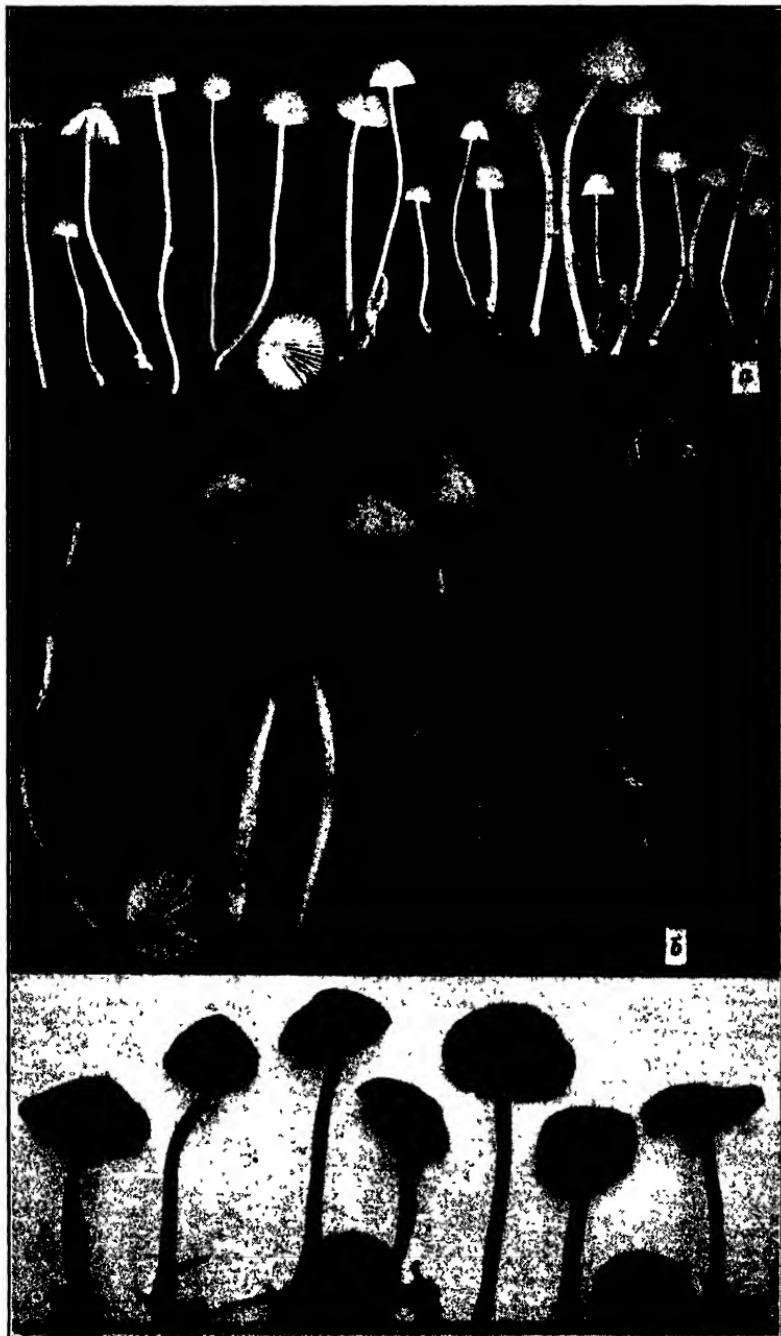


FIG. 1. *a, b, Mycena Clavus; c, Mycena plicosa.*

the cystidia are present on the sides and edges of the lamellae. *Mycena amabilissima* (Peck) Sacc. is the same as *M. Clavus*. The cystidia of the type of *Mycena amabilissima* are present on the sides and edges of the lamellae, fusoid-acuminate, smooth, and measure  $36-63 \times 6-11 \mu$ . The spores are  $5-7 \times 3 \mu$  (very few were found) and the basidia are four-spored. The type collection consists of specimens similar to those shown in figure 1, a. The writer has collected this species abundantly in sphagnum bogs as well as in open oak woods. The colors are delicate and bright but soon fade. At times the disk fades first becoming pure white or creamy and the margin remains bright pink.

**MYCENA ELEGANS** (Pers.) Quél. Champ. Jura Vosg. 221. 1872.

Pileus 5-17 mm. broad, up to 10 mm. high, obtusely conic to expanded umbonate, the margin often recurved in age, "fuscous" on the umbo, paler on the margin (usually grayish olive to whitish), when fading becoming yellowish gray to nearly citron yellow except for the darker disk, often whitish over all, at first with a delicate pruinose covering, soon glabrous, hygrophanous; translucent striate, sulcate when faded; lamellae close to subdistant, narrowly adnate, toothed, seceding in age and at times adhering to each other thus forming a collar around the stipe, narrow to moderately broad, whitish, becoming gray (especially at the base), margin concolorous or pale yellow; stipe 3-6 cm.  $\times$  1-2 mm., pruinose above, strigose and rooting on leaves at the base, somewhat translucent in age, concolorous with pileus or paler, apex usually paler; spores (7)  $8-10 \times 4.5-5 \mu$ ; basidia four-spored; cystidia scattered on the sides and edges of the lamellae, sometimes rare.  $30-40 \times 12-20 \mu$ , clavate, apices obtuse and covered by numerous fine echinulations; odor and taste not distinctive.

The fungus described above has been found regularly every fall in an oak woods near Ann Arbor. The colors were never as bright as those described for *M. aurantio-marginata*, but robust individuals with bright yellow pileus margins have been collected. The gill edges are usually a bright clear yellow but occasionally the color is absent entirely. The fibrils at the base of the stem were white to grayish white. Konrad and Maublanc (7) consider *M. elegans* to be a gray form of *M. aurantio-marginata*.

## MYCENA FAGETORUM Fries, Epicr. Myc. 106. 1838.

Pileus 1–2.5 (3.5) cm. broad, obtusely campanulate when young, soon broadly convex or plane, margin often reflexed in age, "drab" or darker on the apex, margin whitish, becoming "wood brown" to "buffy brown" or "avellaneous" on the disk, in age "mouse gray" to "light grayish olive" and finally fading to pale cinereous or evenly whitish gray, glabrous, lubricous, scarcely hygrophanous, translucent striate when fresh, at times sulcate in age; flesh thick on the disk, thin on the margin, watery gray; lamellae close to subdistant, interspaces usually venose, adnate with a tooth to adnexed, often adhering together when separated from the stipe and forming a collar around it, narrow to broad and often very ventricose, pale to ashy gray, occasionally with a faint incarnate tinge, edge concolorous, in age often stained with sordid brownish spots; stipe 4–7 cm.  $\times$  2–4 mm., "Quaker drab" when fresh and covered with a removable pruinosity, translucent at maturity, paler to nearly white in age, often twisted, rather tough to fragile, white strigose at the base, usually with a long horizontal strigose root under the leaves; odor and taste not distinctive; spores 7–9  $\times$  3–4  $\mu$ , 8–10  $\times$  3–4  $\mu$ , very narrowly pyriform; basidia four-spored, 25–30  $\times$  6–7  $\mu$ ; cystidia on gill-edge only, clavate, with wavy outlines or short obtuse projections covering the apex, 30–35  $\times$  7–10  $\mu$ ; pileus trama homogeneous. Caespitose to scattered among oak leaves late in the fall.

The margin of the pileus is frequently incurved. This led me to search for the species in *Collybia* at first. Ricken's description and illustration fit the Michigan fungus well except that the latter has never been found in a woods where beech trees were present. The species was very abundant in one locality during October 1931 and again in November 1934, but has been only occasionally collected otherwise. The homogeneous pileus and incurved margin indicate a strong relationship with *Collybia* where it perhaps should be placed.

## MYCENA IODIOLENS Lundell, Sv. Vet.-Akad. Skr. 22: 7. 1932.

*Mycena graveolens* Kauffm. & Smith Papers Mich. Acad. 17: 181. 1933.—From the descriptions it is clearly evident that the fungus Lundell described is the same as that described as *M. graveolens*. It is a curious coincidence that the species should be found in the Caucasus Mts. (see notes under *M. psammicola*), in Sweden, and in the United States in the period between 1929 and 1932.

MYCENA LACTEA var. PITHYA (Pers.) Fries—*sensu* Rea, British Basidiomycetae, p. 381. 1922.

Pileus 3–10 mm. broad, convex to obtusely conic, becoming nearly expanded, margin often recurved, usually grayish on the disk and pure white toward the margin, at times pure white or creamy on the disk, sulcate striate, surface dull; lamellae white, very distant, very narrow, bluntly adnate or with a very slight tooth, edge concolor; stipe 10–20 × 0.5–0.7 mm. pure white, evenly pruinose when young, glabrous in age, base inserted on bark but with a few radiating fibers; spores 7–8 × 5–6  $\mu$ , "drop" shaped; basidia two-spored; cystidia on edge only, smooth, 30–35 × 7–10  $\mu$ .

On the bark of a beech log, Warrensburg, N. Y., Sept. 12, 1934. This is a fragile plant with narrow distant gills, otherwise it is similar to the typical form of the species.

MYCENA ODORIFERA (Peck) Sacc. Syll. Fung. 5: 295. 1887.

Pileus 4–10 mm. broad, convex, papillate or center slightly depressed, viscid, glabrous, hardly striate, bluish gray on apex, white on margin, becoming sordid gray or brownish in age, very tough and cartilaginous; lamellae close to subdistant, white to pale gray, adnate but becoming decurrent in age, narrow to moderately broad, tapering to the margin; stipe 1–2 cm. × 1 mm. very cartilaginous, dark gray to bluish gray and covered by a dense rather coarse white pruina, paler and glabrous in age, viscid; spores 7–8 × 3–4  $\mu$ , broadly ellipsoid; basidia four-spored, 30–35 × 5–6  $\mu$ ; cystidia on gill-edge only, filamentose, 38–46 × 6–7  $\mu$ , smooth; pileus covered by a thick gelatinous pellicle, stipe surrounded by a layer of gelatinizing hyphae; odor striking and pleasant; taste none; gregarious or scattered on moss and debris. Apparently a very rare species.

The spores of the type measure 7–8 × 3  $\mu$ . The basidia are both two- and four-spored, and the cystidia on the gill-edge are approximately filamentose in outline, 38–47 × 5–7  $\mu$ . A few were found with forked apices. The gelatinous layer on the pileus is thick, forming about half the cap trama. In the writer's collections the thickness of the pellicle varies considerably. This species was collected at Pinckney and Cross Village in Mich. (33–592 and 33–695), during July and August 1933. The odor somewhat resembles that of *Armillaria caligata* Viv.

## MYCENA PELLICULOSA Fries, Epicr. Myc. 116. 1838.

Pileus 5–10 mm. broad, convex with an abrupt and rather broad depression on the disk, viscid, glabrous, fuliginous, fading to pale gray and at times assuming brownish tints, striae conspicuous, darker than the remainder; lamellae distant, white, arcuate-decurrent, narrow to moderately broad, edge even and concolor, stipe 1–2 cm.  $\times$  1 mm. pallid or concolorous with the pileus, pruinose over all when young, viscid; odor and taste none; spores 7–9  $\times$  4–5  $\mu$  (33–712) or 8–10  $\times$  4–5  $\mu$  (33–706); basidia four-spored; cystidia on gill-edge clavate, roughened with short irregular blunt projections, soon gelatinizing; pileus trama divided into three zones, a surface zone composed of a gelatinizing pellicle  $\frac{1}{2}$  to  $\frac{2}{3}$  the thickness of the entire cap trama, below this a narrow central layer of non-gelatinous interwoven hyphae, the subhymenial zone of gelatinous hyphae and extending to the gelatinous gill-edge (thus the central tissue of the pileus and gill-trama is enveloped on both sides by gelatinizing layers); the stipe has a thick sheath of gelatinizing hyphae. Gregarious on conifer duff and humus under conifers. The entire fruit-body is very tough and cartilaginous.

The above description was drawn from collections 33–706 and 33–712, Cross Village, Mich., Aug. 19, 1933. From the writer's experience this fungus is rare. The species as reported by European investigators seems to be larger and often obtusely umbonate. The gelatinous subhymenium is not mentioned as a character of *M. pelliculosa*, but this could easily have been overlooked as has apparently happened in many studies of *Hygrophorus laetus* Fries. The thickness of the gelatinous layer on the pileus varies greatly depending on the weather conditions and after heavy rains the cap may be nearly dry.

## MYCENA PELTATA Fries, Epicr. Myc. 110, 1838.

Pileus 8–20 mm. broad, convex to obtusely campanulate when young, at maturity occasionally with an obtuse umbo, usually convex to plane, at times with a shallow depression on the disk, at first densely pruinose to frosted, soon glabrous, surface lubricous to subviscid from a very thin adnate gelatinizing pellicle, nearly opaque to faintly striatulate when moist, striae more conspicuous as the colors become lighter, disk usually "fuscous" at first, margin close to "hair brown," in age evenly "drab-gray" or pale cinereous and rather evenly colored; lamellae moderately broad, white to pale grayish at base, adnate with a decurrent tooth, close,

edge concolorous or white; stipe 3–5 cm.  $\times$  1–1.5 mm. (when in deep moss 8–15 cm.), concolorous with pileus or paler, glabrous above, densely white fibrous below, rather tough and cartilaginous, not viscid, with or without a small abrupt bulb at the base; odor and taste not distinctive; spores 8–10  $\times$  3.5–4  $\mu$ ; basidia four-spored; cystidia on the gill-edge only, basidial-like, 28–30  $\times$  6–9  $\mu$ , with wavy outlines or with several finger-like prolongations near the apex; pileus with a region of enlarged cells beneath the cuticle which are filled with a dull brown substance.

On grassy hummocks and on sphagnum, Rees's Bog, Burt Lake, Cheboygan Co., Mich., Oct. 15, 1934 (1157). In consistency this species resembles *M. latifolia* Peck very closely and small forms, in addition, resemble it in color and stature as well. These are easily distinguished by the cystidia however. Those of *M. latifolia* are present on sides and edges of the gills. Those on the sides are fusoid-ventricose in outline with the inflated portion smooth or slightly roughened. Those on the gill-edge are either clavate and roughened or fusoid-ventricose with the enlarged portion roughened by blunt short rod-like projections. Killerman (6) describes our plant well. Ricken (12) p. 442, describes *M. peltata* as having cystidia 40  $\times$  12–15  $\mu$  and so conspicuous that "Durch die Cystiden erscheinen die Lamellen unter der Lupe weissmehlig." Ricken's fungus may possibly be *M. latifolia* which in addition has spores 6–8  $\times$  3–4  $\mu$ .

MYCENA PLICOSA Fries, Syst. Myc. 1: 145. 1821. (FIG. 1, c.)

Pileus 1–2 cm. broad, convex to broadly subconic at first, becoming hemispheric or expanded and broadly umbonate, surface dry, densely pruinose at first, glabrous, blackish when young to dark grayish brown, becoming "drab" to "avellaneous" or dull lead color, somewhat paler to nearly whitish in age, usually dull grayish brown and unicolorous, sulcate striate to "scalloped" on the margin, hardly hygrophanous; lamellae close, narrow to broad, adnate with a tooth, drab to grayish or creamy, faint flesh tint discernable in age, often staining reddish brown where bruised, margin concolorous; stipe 2–5 cm.  $\times$  1–2 mm. densely pruinose, soon polished, often longitudinally twisted striate, rigid and fragile, concolorous with the pileus or nearly white at the apex; narrowed below at the point of attachment or equal, base somewhat white strigose, spores 7–9  $\times$  3.5–4  $\mu$ , basidia four-spored, cystidia 26–30  $\times$  8–11  $\mu$ , capitate to clavate, apex covered by short rod-like

projections; odor and taste none. Gregarious on needle beds under spruce and pine.

In color the Michigan collections differ from Ricken's description but correspond closely to the description of Fries. The fungus varies greatly in stature and in the markings on the pileus. Forms with a "scalloped" margin have been collected as well as forms with plicate pilei. The lamellae in the writer's collections were neither distant nor exceptionally thick for the species in this genus and differ in these respects from the Friesian conception.

**MYCENA PSAMMICOLA** (Berk. & Br.) Sacc. Syll. Fung. 5: 275.  
1887.

Pileus 5–10 mm. broad, broadly campanulate to expanded umbo-nate, "tawny" or with a "russet" cast, "ochraceous-tawny" near the margin and disk "mars brown" when fresh, umbo "fuscous" at times, pale "ochraceous-tawny" to "cinnamon-buff" when faded, edge even, concolorous; stipe 2–5 cm.  $\times$  1 mm., "ochraceous-tawny," apex pallid, equal, glabrous, white fibrous at base, apex pruinose; spores  $7-8 \times 3.5-4 \mu$ ; basidia  $26-28 \times 6-7 \mu$ , four-spored; cystidia on gill edge only,  $20-35 \times 10-12 \mu$ , covered by coarse somewhat pointed projections; odor fragrant, resembling iodoform and developing after the fruit-bodies have been collected, taste none. Gregarious on needle beds under pine and on humus in swampy places.

This species can easily be mistaken for a small *Galerula*. The spore print however is white. Singer (13) places collections with an odor resembling iodoform in *M. psammicola*, but describes the colors as "grau weiss, spores  $9-10 \times 6-6.5 \mu$ ." This is very likely *M. iodiolens* Lundell. The decided brown to ochraceous colors of *M. psammicola* easily separate it from the gray species related to *M. filipes*.

**MYCENA PULLATA** (Berk. & Cooke) Sacc. Syll. Fung. 5: 277.  
1887.

Pileus 5–20 mm. broad, obtusely conic, becoming broadly campanulate to expanded umbonate, colors when young and fresh "army brown," "bone brown," "vinaceous-brown" or "dark purple-drab," fading to "vinaceous-drab" or "natal brown," colors often dull and sordid in age, margin often grayish, at first covered by a faint bloom, glabrous, dry, somewhat sulcate striate near the

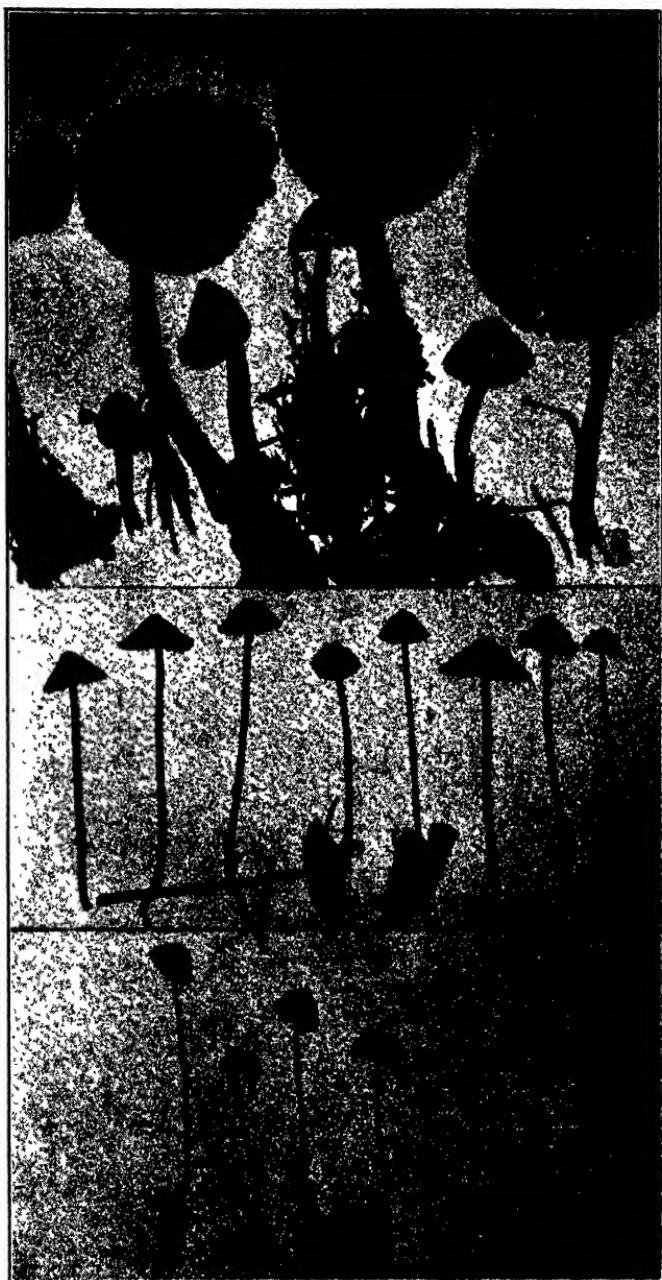


FIG. 2: *a*, *Mycena aetites*; *b*, *Mycena psammicola*; *c*, *Mycena uranii*

margin; flesh rather tough-cartilaginous; lamellae narrow to moderately broad, close to subdistant, narrowly adnate, whitish cinereous or vinaceous drab, usually concolorous with the pileus in age; stipe  $4-15 \times 1-2$  mm. very rigid-cartilaginous, "purple-drab" to "vinaceous-drab" or "vinaceous-fawn" often grayish below, faintly fibrous pruinose above, usually longitudinally fibrous striate but glabrous and polished at times, white mycelioid at base; spores  $8-10 \times 6.5-7.5 \mu$ ,  $8-10 \times 5-6 \mu$ ,  $8-11 \times 5.5-6.5 \mu$  or  $9-11 \times 5-6 \mu$  in different collections, ellipsoid; cystidia on gill-edge smooth or with finger-like prolongations, fusoid-ventricose to clavate before branching,  $25-35 \times 7-9 \mu$ ; odor and taste not distinctive.

Whether or not the fungus described above is properly placed in *M. pullata* is a matter of opinion. The dark colors with a purplish brown cast and the stature as shown in Cooke's illustration (4) indicate a very close relationship to the Michigan collections. Atkinson (1) considered this fungus to be the American form of *M. polygramma* chiefly because of the longitudinal fibrous striations which may be present on the stipe. Specimens of the latter species from Europe in the Atkinson collections and from England in the University of Michigan Herbarium do not satisfactorily confirm this disposition of the fungus. The colors of *M. pullata* as described here are strikingly different from those usually attributed to *M. polygramma* and have been observed to be constant in their range of variation during the past five seasons. The species grows singly among the leaves and on humus in oak woods around Ann Arbor, and has almost invariably been found in company with *Mycena vitilis*.

**MYCENA URANIA** Fries, Syst. Myc. 1: 144. 1821. (FIG. 2, c.)

Pileus 4-10 mm. broad, conic to hemispheric, occasionally broadly umbonate, usually convex in age, "dark plumbeous" or "deep Varley's gray" when fresh, dark bluish gray colors quite pronounced, fading through "lilac-gray" or violet grays and finally pale drab, margin slightly paler when fresh, with a hoary sheen at first, glabrous and dull in age, rather dry, margin more or less sulcate striate and somewhat crenate at times; lamellae narrow, subdistant to distant, broadly adnate to uncinate-adnate, white or whitish when young, becoming bluish or pallid gray, edge concolorous; stipe 1-3 cm.  $\times$  0.5-1 mm. "pale violet-gray" to "deep Varley's gray," becoming paler, dry, with a hoary unpolished appearance at first, the apex slightly pruinose, rigid, tubular; spores

$7-9 \times 4-5 \mu$ , ellipsoid; basidia four-spored,  $20-25 \times 6-8 \mu$ ; cystidia present or absent on the sides, numerous on the edge,  $26-30 \times 8-10 \mu$ , clavate, the apex covered by short rod-like processes; odor and taste none. Gregarious on wet swampy ground among mosses and decaying leaves during the late summer and fall. Apparently a very rare species in Michigan.

Fries described the stem as flaccid and the gills as white. In my collections the stems were usually rigid enough to hold the small caps upright although fruit-bodies from mossy places were often rather decumbent. In addition the gills were always dark. Differences such as these however, have been observed in individuals of single collections of many other species and are not considered sufficient to separate the American collections. The microscopic details of the species are very poorly known, and are omitted in most of the descriptions. *M. atrocyanea*, which it apparently resembles in color, is ordinarily classed in the fragile group. If Ricken's interpretation of the latter is correct *M. urania* is easily separated from it by the clavate roughened cystidia.

MYCENA VITILIS Fries, Epicr. Myc. 113. 1838.

Pileus 5-10 mm. broad, conic, campanulate or nearly expanded and somewhat umbonate, "drab" when faded, brownish colors more evident when fresh, striate, subviscid to lubricous when moist but pellicle very thin and adnate, glistening when dry, at times unpolished, glabrous, margin even; flesh rather leathery in consistency; lamellae close, narrow, equal, white or grayish, attached by a tooth to narrowly adnate, edge concolor and often slightly eroded; stipe 6-12 cm.  $\times$  1-1.5 mm., bluish black at first, soon gray, nearly concolorous with the pileus in age, apex somewhat fibrous striate at first, tough, flaccid in age, strigose at the base and rooting in the debris, often attached to small sticks; spores  $9-11 \times 5-6 \mu$ ; basidia four-spored; cystidia occasionally on the sides, frequent on the edges of the lamellae,  $30-40 \times 8-14 \mu$ , fusoid-ventricose or with several contorted prolongations.

Not much concerning the occurrence of this species in the United States seems to be known. It has been found rather abundantly late in the fall (October and November) in the oak woods around Ann Arbor. Lange's (8) description and comments describe the Michigan collections accurately. It is nearly always attached to small sticks buried in the leaves and debris, and the stem is usually

very crooked. When the weather conditions are exceptionally favorable robust forms are found occasionally which are strict and rigid in their appearance. The consistency is decidedly more cartilaginous than that of *M. filipes*, and the cystidia separate the two readily.

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# NOTES ON SOME SPECIES OF THE UREDINALES<sup>1</sup>

GEORGE B. CUMMINS

(WITH 5 FIGURES)

The miscellaneous notes which follow in this paper have been assembled from time to time as interesting collections have been received or old collections restudied. They include new records for the United States, new species, new combinations and revised descriptions. All specimens, including types, are deposited in the Arthur Herbarium of this institution.

## UREDO DIOSCOREAE-ALATAE Racib.

While studying a collection of rusts on some species of *Dioscorea* from the Philippine Islands sections were made of specimens of this species as represented in the Arthur Herbarium. One specimen was collected by P. W. Graff at Manila, Luzon, P. I., Dec. 2, 1912, and issued by Sydow in his *Fungi Exotici* as no. 230. The other was collected at Bangai village, Samoa, June and July 1926, by H. E. Parks as no. 8558. Both are on *Dioscorea alata*.

The uredia in Graff's collection are deep in the tissue of the leaf, apparently located about midway between the upper and lower epidermis and thus agree with the description given by Raciborski (13) for *Uredo Dioscoreae-alatae*. In Park's collection the uredia are just beneath the lower epidermis. The urediospores correspond closely in both collections and despite the difference in the position of the sori the two collections certainly belong to the same species.

Highly gelatinized telia (FIG. 1, 2A) are present in abundance in both collections. These telia are subepidermal in origin and show young spores almost as soon as the development has progressed to a point where the primordial sorus can be differentiated

<sup>1</sup> Contribution from the Botany Department, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

from the host tissue. The gelatinous matrix in which the spores are imbedded appears to be present from the beginning, and as soon as the epidermis ruptures, it protrudes above the surface of the host as a hyaline umbo. From this it appears probable that the epidermis is ruptured by the swelling of the matrix, because the delicate teliospores not only appear to be inadequate for such a task but are seldom pressed against the epidermal cells. Once the epidermis is ruptured the telia spread laterally to a considerable extent and often unite with other sori to form a nearly continuous telial layer 200 to 300  $\mu$  or more in extent. The upturned epidermis usually remains visible between such groups of telia.

The teliospores are cylindrical, straight or more or less sinuous and complete their development within the gelatinous matrix, only the basidiospores being liberated above its surface. Details at the base of the sorus are difficult to see but apparently more than one teliospore is produced from a single basal cell by lateral budding (FIG. 2A). In this way several generations of teliospores in different stages of development are present in a single sorus. The germinated spores collapse, making space for succeeding generations of spores.

Germination takes place by the formation of an internal basidium, *i.e.*, the teliospores become 4-celled by the development of septae, and each cell then produces a sterigma which proceeds upward through the gelatinous matrix and liberates a single basidiospore above the surface of the matrix. The teliospores are somewhat variable in size, measure 8–10 by 46–60  $\mu$  and have a smooth, hyaline wall, less than 1  $\mu$  in thickness.

The characters given here for the telia of this species correspond so closely with those of the genus *Goplana*, as described by Raciborski (13), that I have no hesitation in transferring the species to that genus. Only three species, all from Java, have previously been included in the genus. Uredia have not been described for any of the three.

This fungus was first described by Berkeley and Broome (4) as *Aecidium Dioscoreae* and later transferred to *Uredo* by Petch (11), who pointed out that it was apparently identical with *U. Dioscoreae-alatae* Racib. (*l. c.*). Petch's transfer was not valid since Henning (8) had previously used the name for a *Uredo* now con-

sidered to be a synonym of *Sphenospora pallida* (Wint.) Diet. Since *Aecidium Dioscoreae* Berk. & Br. is the first name applied to this fungus and since the name was given to the diploid or per-



FIG. 1. This photograph was taken of a freehand section of a telium of *Goplana Dioscoreae* (from Sydow's *Fungi exotici* 230, issued as *Uredo Dioscoreae-alatae* Racib.). The general characters of the telium are shown with the teliospores developing upward in the gelatinous matrix. No germination has occurred. (See also fig. 2A.) X about 500.

fect state the specific name becomes available when placed in a genus other than the form-genus *Uredo*, and is not affected by Hennings' name which was given later. This usage is in accord with Arthur's (3) interpretation of the rule. Accordingly the name and synonymy of this species is as follows: **Goplana Dioscoreae** (Berk. & Br.) comb. nov. (*Aecidium Dioscoreae* Berk. & Br., Jour. Linn. Soc. Bot. 14: 95. 1875; *Uredo Dioscoreae-alatae* Racib. Paras. Algen und Pilze Javas I, 29. 1900; *Uredo Dioscoreae* Petch, Ann. Roy. Bot. Gard. Peradeniya V. 4: 252. 1912, not *Uredo Dioscoreae* Henn. Hedwigia 35: 255. 1896.

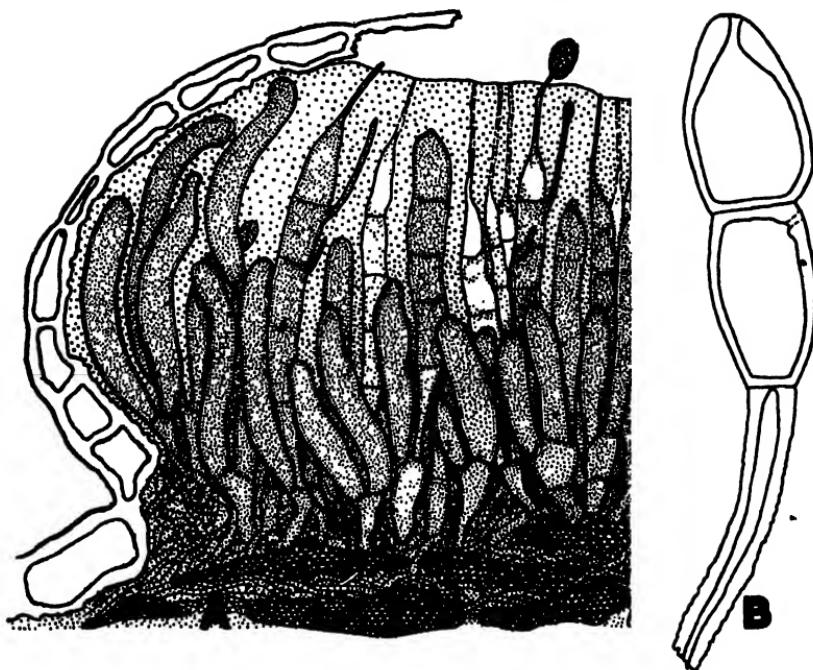


FIG. 2. A. This camera lucida drawing represents a telium of *Gopala Dioscoreae*. The spores are imbedded in a gelatinous matrix, germinate by an internal 4-celled basidium, produce sterigmata which grow through the matrix and produce one basidiospore each above the surface of the matrix. Germinated teliospores collapse and are followed by succeeding generations of new spores as shown. More than one spore apparently comes from a single basal cell in the manner shown. The dark areas at the base of the sorus represent collapsed host-tissue. B, a teliospore of *Puccinia Parksiana*. The teliospores in this rust are a bright golden-brown and have thick-walled pedicels concolorous with the walls of the spores.  $\times$  about 650.

#### *Puccinia Parksiana* sp. nov.

Teliis hypophyllis, rotundatis, compactiusculis, rufobrunneis, circinnatis in maculis 4–10 mm. diam.; teliosporis (TEXT FIG. 2B) oblongis, 22–26  $\times$  55–80  $\mu$ , apice et basi rotundatis, medio constrictis; membrana 2–3  $\mu$  cr., apice incrassatis 4–6  $\mu$ , aureo-flavidis, levis; pedicello concolori, persistenti, usque 60  $\mu$  longo 13  $\mu$  crasso.

On *Smilax vitiensis* Seeman, near Suva, Fiji, May 1926. H. E. Parks 8500.

No other spore-form is present in this material and there is no doubt that the species is microcyclic. The telia are arranged in from three to five concentric rings. The teliospores resemble those

of *Puccinia Smilacis-chinae* P. Henn. and *P. Merrillii* P. Henn. in shape and color but differ in having non-inflated pedicels.

The species is named in honor of the collector, Mr. H. E. Parks of Trinidad, California, who is at present contributing important information regarding the rust-flora of northern California in addition to his earlier collections of such exotic species as the one described above.



FIG. 3. *Puccinia liberta* Kern. The photograph shows the subepidermal, locular, paraphysate telia present in the type specimen (on *Eleocharis* sp., Grènada, dept. Grenada, Nicaragua, Feb. 11, 1903. C. F. Baker 2385).

#### PUCCINIA LIBERTA Kern

Telia of this species have seldom been collected but they were found to be plentiful in a specimen on *Eleocharis palustris* (L.) R. & S. collected by H. E. Parks at Dry Lagoon, Humboldt Co., Calif., Sept. 1934. In studying the telia in section it was found that the teliospores are formed in subepidermal locules surrounded by paraphyses. No such structures were mentioned by Kern (9) when he described the species but after sectioning telia present in the type collection (on *Eleocharis* sp., Grenada, dept. Grenada, Nacaragua, Feb. 11, 1903. C. F. Baker 2385) it was found that paraphyses are also present in the type (FIG. 3).

## UROMYCES SCIRPI (Cast.) Burr.

*Uromyces Scirpi* as represented in the Arthur Herbarium and as published by Arthur (1, 2) is composed of two clearly defined species, separable by morphological characteristics of both the urediospores and the telia. These differences came to my attention while studying a specimen on *Scirpus californicus* (Mey.) Britt. collected by H. E. Parks (no. 5156) at Dry Lagoon, Humboldt Co., Calif., Sept. 14, 1934. This specimen differed from published descriptions of *U. Scirpi* in having larger urediospores with more germ pores, indehiscent, substomatal, loculate telia surrounded by brown paraphyses and longer teliospores with pale, thin walls and short pedicels. The differences in the appearance of the telia of the two species is shown in the photographs taken of free-hand

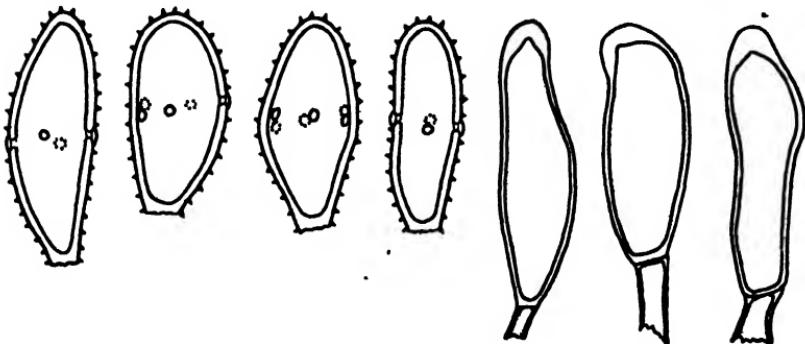


FIG. 4. Urediospores and teliospores of *Uromyces americanus* Speg. Drawn with the aid of a camera lucida from type material (near Mendoza, Argentina, January 1908. C. Spegazzini). X about 650.

sections and reproduced in Figure 5: A is from Spegazzini's type of *Uromyces americanus*, B is from a specimen of *U. americanus* on *S. validus* from Wisconsin, and C is from a specimen of *U. Scirpi* on *S. campestris* from North Dakota.

A study of all of the material assigned to this species in the Arthur Herbarium proved that this form was abundantly represented. No published description was found which could apply to this rust. However, a specimen collected on *Scirpus americanus* near Mendoza, Argentina in January 1908 by Spegazzini proved to be identical when studied in section. This specimen was published by Spegazzini (14) as *Uromyces? americanus* Speg. (n. sp.).

Spegazzini's description makes no mention of the loculate character of the telia and describes the urediospores as globoid. Since the specimen which I have studied is a part of Spegazzini's type (sent to Dr. J. C. Arthur by Spegazzini in September 1920) it has been possible to detect the inadequacy of his description and to establish the fact that *U. americanus* Speg. is a valid species represented in the flora of both North and South America and easily distinguished from *U. Scirpi* (Cast.) Burr. Petrak and Sydow (12) have also pointed out the fact that *Uromyces americanus* is to be considered as distinct from *U. Scirpi* and found in addition that *Macrophoma americana* Speg. is a rust and was described from telial material of *U. americanus*.

A redescription of the species together with the hosts and distribution follows:

Uredia on the stems, subepidermal, covered by the epidermis and opening by a longitudinal slit, oblong, 0.5–1 mm. long, cinnamon-brown; urediospores (FIG. 4 *from type*) variable in size and shape, oblong, ellipsoid or spindle-shaped, 15–25 × 31–50  $\mu$ ; wall 1.5–2  $\mu$  thick, pale cinnamon-brown, finely echinulate, the pores 4–6, equatorial, distinct. Telia (FIG. 5, A, *type*; B, *Wis.*) on the stems, in blackish spots of varying size, subepidermal, indehiscent, loculate, round, 50–70  $\mu$  in diameter, surrounded by subepidermal paraphyses; teliospores (FIG. 4 *from type*) variable, oblong or cylindric, 14–21 × 39–65  $\mu$ ; wall 1  $\mu$  thick at sides, 3–5  $\mu$  above, yellowish or nearly colorless, smooth, the pore apical; pedicel short, hyaline or tinted.

On *Scirpus americanus* Pers., Ala., Del., Tex.; Bermuda; Argentina.—*S. californicus* (Mey.) Britt., Calif.—*S. validus* Vahl. Ind., Neb., Wis.; N. S., Ont.

No cultures have been reported for this species.

The segregation of *U. americanus* from *U. Scirpi* necessitates some revision of the description of *U. Scirpi* given in the N. Am. Flora and Arthur's "Manual." The telia (FIG. 5, C) were found to have a few paraphyses but are always erumpent and dehiscent. The teliospores measure 14–22 by 26–50  $\mu$ . *Scirpus americanus* and *S. validus* should be omitted from the list of hosts. The records for *S. americanus* from Indiana and Wisconsin should be credited to *S. fluviatilis*. *Uromyces? americanus* Speg. should be removed from the synonymy.



FIG. 5

## UREDO ERICAE Naumann

In 1934 Diehl (6) reported a rust on *Erica* sp. (*E. hyemalis* Nichols), collected by G. L. Stout at Colma, Calif., July 12, 1934, as *Uredo Ericaë* Naumann. Diehl's identification was tentative since he had no material for comparison, and at the time that it was sent to this laboratory for verification no material was available in the Arthur Herbarium. Since then a fragment of *U. Ericaë* has been received from Dr. G. Samuelsson of the Naturhistoriska Riksmuseet, Stockholm which was collected at Leipzig, Germany, in 1913 on *Erica gracilis* by Naumann.

Free-hand sections proved that *U. Ericaë* has a well developed cellular peridium which was not included in the description given by Naumann, although he mentions, in his general discussion, that he observed a peridium-like structure in sections of the sori. In this respect *U. Ericaë* and the collection from California agree. The urediospores also correspond, being ellipsoid and having eight scattered pores in an echinulate, colorless wall  $1.5 \mu$  thick. I find no characters which would warrant a separation of the two collections.

Diehl also pointed out that the rust from California was apparently identical with *Thecopsora Fischeri* Cruchet, after comparing it with material from Spain. Dr. Cruchet kindly sent me two collections of his fungus and through the kindness of Dr. Sydow I have also been able to examine Cruchet's type collection. The fungus has a peridium as described and illustrated by Cruchet (5). For a more accurate description and illustration see also González Fragoso (7).

A few hyaline thin-walled paraphyses are present at the base of the sorus inside of the peridium in *U. Ericaë*. Such paraphyses were not seen in *T. Fischeri* but were found in the California specimen. These structures might easily be overlooked and do not warrant separate specific names for these two fungi since the urediospores and the peridium are the same in both.

Because of the morphological characteristics of the uredia *Uredo Ericaë* is placed in the genus *Pucciniastrum* as *P. Ericaë* (Naumann) comb. nov. with the synonymy as follows: *Uredo Ericaë* (Naumann, Jahresb. Vereinig. angew. Bot. 9: 207. 1912; *The-*

*copsora Fischeri* Cruchet, Bull. Soc. Vaud. Sci. Nat. 51: 77. 1916).

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#### EXPLANATION OF FIG. 5

FIG. 5. The photographs were all taken of free-hand sections at a magnification of about 150 diameters. A, the loculate telia present in the type material of *Uromyces americanus* Speg. (on *Scirpus americanus* near Mendoza, Argentina, January 1908. C. Spegazzini); B, telia of *U. americanus* (on *Scirpus validus*, Madison, Wis., June 6, 1907. E. W. Olive); C, telia of *Uromyces Scirpi* (Cast.) Burr. (on *Scirpus campestris*, Kulm, N. D., Sept. 1909. J. F. Brenckle 326, Brenckle Fungi Dakot. 120).

## NOTES ON SOME FUNGI FROM COLOMBIA

F. D. KERN AND R. A. TORO

In the spring of 1934 the writers spent some time in South America, chiefly in Venezuela, making mycological explorations and collecting specimens. While spending some time in the State of Tachira, Venezuela, the nearness to the Colombian border tempted us to cross over and do a bit of collecting there. The specimens here reported were collected in the vicinity of Cucuta, Department Norte de Santander, Colombia, on May 11, 1934.

Our knowledge of the fungous flora of Colombia is well presented in a paper entitled "Mycological Explorations of Colombia" by Carlos E. Chardon and Rafael A. Toro, Journal of the Department of Agriculture of Puerto Rico 14: 195-369, 1930. Further information regarding the Uredinales there is to be found in an "Annotated Index of the Rusts of Colombia" by F. D. Kern, H. W. Thurston, Jr., and H. H. Whetzel, Mycologia 25: 448-503, 1933.

We are indebted to the administrative officers of the University of Puerto Rico, especially Chancellor C. E. Chardon, for making possible our trip to South America and to various officials and friends there for assistance and courtesies which were accorded to us. We acknowledge also with thanks the aid of several phanerogamic botanists in identification of hosts.

**PHYLLACHORA MACHAERIICOLA** (P. Henn.) Th. & Syd. Ann. Myc.  
13: 504. 1915.

*Physalospora machaeriicola* P. Henn. Hedwigia 43: 243. 1904.

A very common species on this host in Venezuela. Reported here for the first time from Colombia.

On *Machaerium Humboldtianum* Vogel, Kern & Toro no. 1.

HYOSPILINA OSPINAE (Chardon) Chardon & Toro, Monog.

University of Puerto Rico, series B, 2: 192. 1934.

*Gnomonia Ospinae* Chardon, Bol. Real Soc. Esp. Hist. Nat. 28: 120. 1928.

On *Tecoma pentaphylla* Juss., Kern & Toro no. 4.

**Septoria cicutana** sp. nov.

Maculae laxae vel densiuscule per folium dispersae, in utraque pagina visibles, ambitu orbiculares v. irregulares, primitus griseobrunneae, dein pallescentes albidae, linea marginali obscure rufobrunnea plerumque valde elevata acutissime definitae, .5-2 mm. diam.; pycnidia epiphylla, sat regulariter densiusculeque per macula dispersa, depresso-globosa, 90-110  $\mu$  diam., ostiolo tantum plano papilliformi poro rotundo aperto punctiformiter erumpentia; pariete membranaceo, contextu subhyalino, dilute griseo-brunneolo fibroso; conidia filiformia, utrinque plerumque leniter attenuata, obtusa, vermicularicurvata, hyalina, 34-40  $\times$  .8-1  $\mu$ .

In foliis *Tecoma pentaphyllae* Juss., Kern & Toro no. 5.

**CEROTELIUM DESMIUM** (Berk. & Br.) Arth. N. Am. Flora 7: 698. 1925.

*Uredo Gossypii* Lagerh. Jour. Myc. 7: 48. 1891.

On *Gossypium peruvianum* Cav., Kern & Toro no. 3.

A common species wherever *Gossypium* grows. Previously collected in Colombia both by Mayor and Chardon.

**PUCCINIA CENCHRI** Diet. & Holw.; Holw. Bot. Gaz. 24: 28. 1897.

On *Cenchrus echinatus* L., Kern & Toro no. 6.

**PUCCINIA OBLIQUA** Berk. & Curt.; Berk. Jour. Linn. Soc. 10: 356. 1869.

On *Mesechites trifida* (Jacq.) Muell. Arg., Kern & Toro no. 9.

The use of this name for a rust on *Mesechites* which belongs to the family Apocynaceae is somewhat doubtful. Up to this time *Puccinia obliqua* has been used only in connection with hosts belonging to the Asclepiadaceae. There is also a question whether *P. obliqua* has a limited or systemic mycelium or both. The name has been used by several authors for microcyclic forms which are often systemic, with pedicel usually attached obliquely, and with

mesospores intermixed. The specimen here recorded is of this type and is practically indistinguishable from numerous West Indian and South American specimens on various members of the family Asclepiadaceae.

**PUCCINIA ROTUNDATA** Diet. *Hedwigia* 36: 32. 1897.

On *Vernonia brasiliiana* (L.) Druce, *Kern & Toro* no. 7.

A common rust in Colombia already reported on this species of host and two or three other species of the genus *Vernonia*.

**UROMYCES HEDYSARI-PANICULATI** (Schw.) Farl.; Ellis, N. Am. Fungi 246. 1879.

*Uredo amagensis* Mayor, Mem. Soc. Neuch. Sci. Nat. 5: 584. 1913.

On *Desmodium scorpiurus* (Sw.) Desv., *Kern & Toro* no. 8. This is a new host for Colombia.

**UROMYCES PROEMINENS** (DC.) Pass. Rab. Fungi Eur. 1795. 1873.

*Uromyces euphorbiicola* Tranz. Ann. Myc. 8: 8. 1910.

On *Chamaesyce hypericifolia* (L.) Millsp., *Kern & Toro* no. 2.

While this rust is common on this species of host in the West Indies, this is apparently the first report of it from Colombia on this host. It has been reported on *C. brasiliensis* and *C. hirta*.

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# THE ROLE OF INTRACELLULAR MYCELIUM IN SYSTEMIC INFECTIONS OF RUBUS WITH THE ORANGE-RUST<sup>1</sup>

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(WITH 42 FIGURES)

It is well known that infections arising from basidiospores of either the short cycled aeciospores or the long cycled teliospores of *Gymnoconia interstitialis* become systemic and perennial. The orange-rust has perhaps been studied more extensively than any other rust possessing this type of infection. From the researches of Dodge (5, 6, 7, 8), and the earlier experiments of Kunkel (12), the following summary of our present knowledge regarding systemic infections may be made: Basidiospore infections from both the short and the long cycled strains become systemic when made upon young shoots of *Rubus* as they push through the ground in the spring; the mycelium establishes itself in the cambium and phloem and grows downward into the root crown; new shoots arising from the crown the following year will be rusted. A stimulation of shoot production each year results in a typical witches' broom. The mycelium is systemic and generally confined to the pith, except in the growing regions; shoots over 6 inches in height when inoculated become only locally infected since the mycelium is unable to reach the roots.

Further information is highly desirable, particularly as to the early stages of infection. While it is known where and when infection takes place, nothing is known of the actual method of penetration, the relation of the mycelium to the host cells and its method of development and distribution during the first year. In this paper the history of the infection has been followed in detail

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from the time of inoculation to the appearance of the rust the following year.

The inoculum was obtained from plants collected in the vicinity of New York, and by germination tests was shown to be clearly of the short cycled strain. The strain germinating with a two-celled promycelium was used in most cases, although the four-

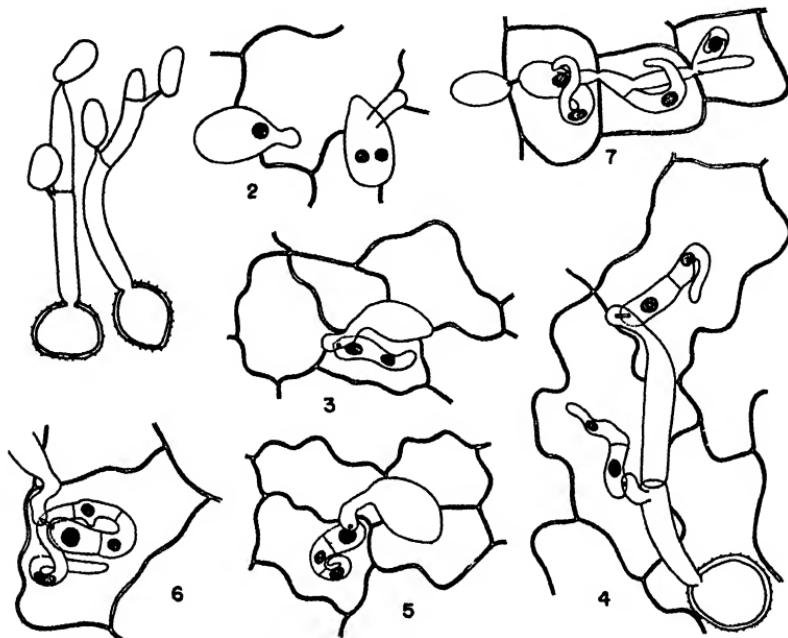


FIG. 1, typical germination of short cycled aeciospores on agar; 2, germinating basidiospores on leaf; 3, young penetration hypha in epidermis; 4, penetration directly from the promycelium after 2 days; 5-6, penetration hyphae of three and four cells; 7, young intracellular mycelium arising from a branch of the penetration hypha after 4 days. (Fig. 1 reduced to 400 X, all other figures reduced to 600 X.)

celled strain was also used and found to behave similarly. In order to obtain shoots for a series of inoculations, portions of roots from known healthy plants were layered in sand. When the shoots were about half an inch in length the spores were lightly dusted on with a camel's hair brush. At various intervals material was fixed for detailed study but in the main the results here presented are from freehand sections of fresh material. In a few cases leaves were inoculated in petri dishes as described in an earlier

paper (13). Penetration takes place readily on very young leaves, but as the leaf begins to mature penetration is accomplished only with great difficulty. This fact proved to be of considerable value since "in toto" preparations could be made from young inoculated leaves and the details of penetration and early mycelial development readily studied from them.

#### DEVELOPMENT OF THE INTRACELLULAR MYCELIUM

When sown upon young leaves in petri dishes or upon shoots kept in a moist chamber, germination takes place readily and the promycelia are fully formed within twenty-four hours (FIG. 1). As the basidiospores mature they are shot from the sterigmata and usually could be located a short distance away. Germination follows immediately and a short germ tube is formed (FIG. 2). Nuclear division sometimes occurs before penetration and the spores become binucleate (FIG. 2). From the rounded tip of the short germ tube a fine tube-like process is formed which passes through the cuticle and cell wall and enters the underlying host cell. The entire contents of the spore move through this opening and a short intracellular hypha begins to develop. The nucleus moves in and divides and the two nuclei can be readily seen (FIG. 3). This intracellular strand, the penetration hypha, continues to grow and the end usually becomes somewhat coiled.

An interesting feature of penetration by this short cycled rust is found in the behaviour of the sterigmata of some promycelia. As shown in figure 4, the sterigmata become rounded resembling the germ tubes of the basidiospore, and from this rounded part the penetration tube pushes out. In the upper cell the details of this process may be seen very clearly. This method of penetration seems to take place when the promycelium happens to lie in close contact with the epidermis, and is not infrequently encountered in inoculations made on leaves.

The penetration hypha grows slowly in the epidermal cell and the number of nuclei also increases. In the stage shown in figure 5 three nuclei may be readily observed. In these "in toto" preparations it is often difficult to see the cross walls of the hyphae, although the nuclei are very distinct. Further growth results in the formation of a characteristic penetration hypha (FIG. 6). The

basal cell is much enlarged and has a rounded outline. The second and third cells are narrower and the latter is sending out a branch. The terminal cell is becoming coiled in the epidermal host cell.

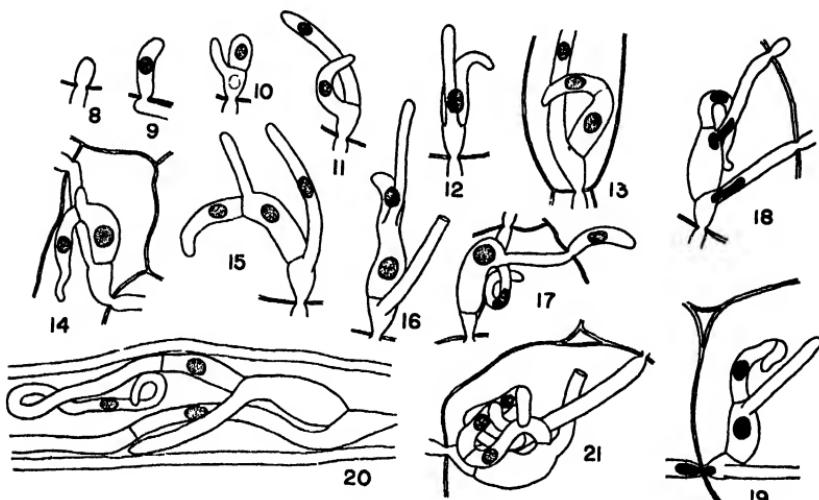


FIG. 8-17, development of intracellular mycelium showing stages in the formation of the runners and the terminal coil (see text for details); 18, nuclei beginning to move into the runners, secondary runner having just entered the adjacent cell; 19, nucleus in hypha passing through the opening in the cell wall; 20, intracellular mycelium in vascular tissue; 21, typical mycelium in pith after 100 days (note the compact multicellular terminal coil), runners practically empty. (All figures reduced to 600 X.)

Up to this point the details of penetration agree very closely with the many accounts of basidiospore infection in the rusts. According to all of these accounts the penetration hypha gives rise immediately to an *intercellular* mycelium. In *Gymnoconia*, however, the penetration hypha sends out branches which enter adjacent cells to form a peculiar *intracellular* mycelium. From the basal cell of the penetration hypha a branch arises which grows to the side wall of the epidermal cell, forms a narrow opening and pushes through to the next cell. The intracellular mycelium which is thus initiated is characteristic in that a regular and definite hyphal system is formed in each cell entered. Figure 7 shows that the branch arising from the penetration hypha which has entered the next cell, has itself branched and given rise to a coiled hypha remarkably like the original penetration hypha. In the third cell,

which in this case is the limit of the intracellular development, the hyphal system is similar to that of the first two cells. It has been found that in every cell entered by this mycelium a similar development takes place, and this hyphal complex is so constant and characteristic that it may be readily recognized even in the presence of the hypha-like haustoria of the intercellular mycelium.

Various stages in the development of this mycelium are shown in detail in figures 8 to 17. These drawings show intracellular mycelium as found in different tissues and resulting from various inoculations. In figure 8 a young hypha has just entered a host cell. The opening is easily seen and there is a well marked but not extreme constriction at that point. This constriction is one of the characteristic features of this mycelium. The nucleus is near the tip and slips through the opening (FIG. 9, 17). Nuclear division takes place and a wall is laid down cutting off the tip. From the sub-terminal cell a branch arises (FIG. 10). For convenience the hypha so formed will be termed the *primary runner*. As growth continues the primary runner elongates rapidly, with the nucleus remaining in the tip, while the terminal cell elongates and bends either to one side or the other (FIG. 11). This stage is also shown in figure 12. The basal part of the terminal cell becomes much enlarged especially when compared with the primary runner (FIG. 12, 13). In the next stage the nucleus of the terminal cell divides (FIG. 13) and the enlarged terminal cell, now sub-terminal, sends out a branch just below the cross wall (FIG. 14). This branch is similar to the primary runner and will be referred to as the *secondary runner*. The secondary runner elongates rapidly (FIG. 15, 16) and pushes on into the next cell (FIG. 17, 18). In one or two cases this runner has been observed to branch before reaching the cell wall. The terminal cell also continues to grow but turns back upon itself forming a loose loop (FIG. 17). The nuclei, as they begin to move out into the runners, are often considerably attenuated (FIG. 18); once in the runners, however, they assume a more oval outline (FIG. 15, 17). Occasionally a nucleus is found in the process of passing through the opening in the cell wall. In figure 19 the nucleus has just commenced the passage and shows a well marked constriction.

It may be said then that at first the hypha in the cell is three-

celled; from the first cell arises the primary runner; from the second, which is characteristic in shape and size, the secondary runner is formed, while the third, or apical cell continues to grow slowly forming a rather complicated coil (FIG. 20, 21). When the runners enter the next cell each will give rise to a similar struc-

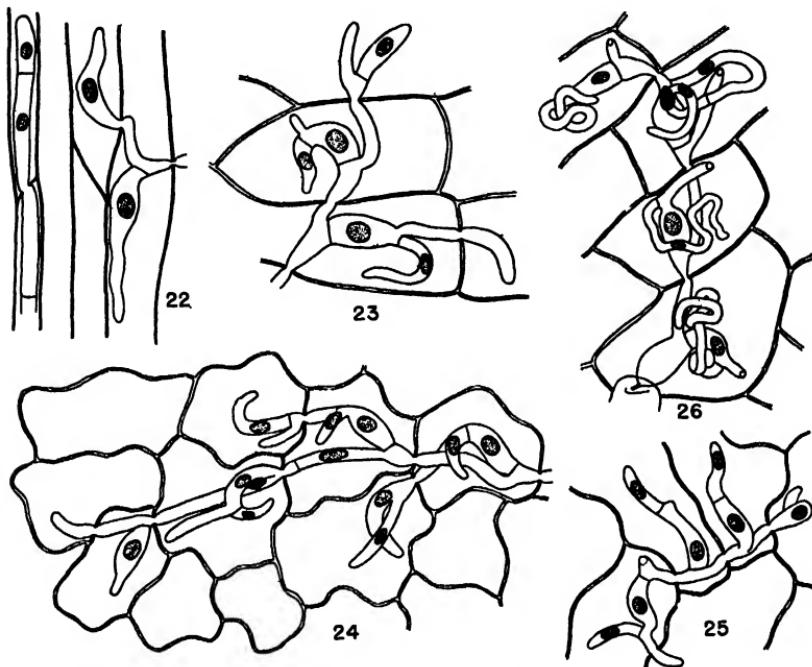


FIG. 22, young intracellular mycelium in phloem; 23, typical mycelium in cortex showing origin and development of the runners; 24, intracellular mycelium in epidermis of inoculated leaf; 25, mycelium in epidermis originating from a runner which entered from the palisade; 26, intracellular mycelium in epidermis showing infection hypha and part of the four-celled promycelium from which it arose; note coiling of tip and runners entering the leaf tissues below (indicated by a small circle), after 10 days. (All figures reduced to 600 X.)

ture (FIG. 17). In figure 20 is shown a typical intracellular mycelium in the vascular region. The primary runner has advanced into an adjacent cell. The secondary runner has grown but a short distance, while the terminal coil has become two-celled and is greatly elongated. In older intracellular mycelia the two runners may appear to be almost empty while the apical unit is multicellular and much coiled (FIG. 21).

In figures 22 to 26 various hyphal strands of different ages and from different tissues are shown. The first two figures represent young hyphae in longitudinal section, figure 22 being from the vascular tissue and figure 23 from the cortex. The remaining figures represent various stages in leaf infections. If any of these strands be studied closely it will be at once apparent that one can interpret the mycelial structure in any one cell in terms of the units shown in figures 8 to 18. The mycelium which becomes established in the leaves is exceptionally favourable for study, since long hyphal strands are formed running from cell to cell in the epidermis (FIG. 24, 25, 26). The details of the formation of the primary and secondary runners may be readily observed in these figures. In the leaf all tissues are invaded, the mycelium travelling through the palisade and mesophyll and into both the upper and lower epidermis. In figure 25 a runner has entered the upper epidermis from the palisade and is growing in characteristic fashion. Figure 26 represents a hyphal complex in the epidermis arising from infection by a single promycelium, part of which is shown in the lower part of the drawing. From each of the four epidermal cells invaded by the mycelium, runners have entered the palisade layers below; such points are indicated by small circles in the drawing.

The distribution of the mycelium and the rate of growth was studied by making freehand sections of inoculated shoots at various intervals. At the end of five days the mycelium has invaded the cortex and has almost reached the scattered bundles. This is represented diagrammatically in figure 27; at *A* is a diagram of an inoculated shoot; *B* represents the same shoot 5 days later; at *C* is a cross section of the infected shoot drawn with camera lucida. The distribution of the mycelium is indicated by the small circles. In the cross section, *C*, the mycelium is well established in the cortex and has almost reached the young bundles. By the tenth day sections show that all parts of the vascular bundle have been invaded. Twelve days from the time of inoculation mycelium is abundant in the cortex, bundles and pith (FIG. 28). In the cross sections of this stage (FIG. 28*B*) the distribution of the mycelium suggests that infection has taken place at two major points on the shoot. From these points the mycelium has spread somewhat fan-wise so that approximately one half of the bundles are infected.

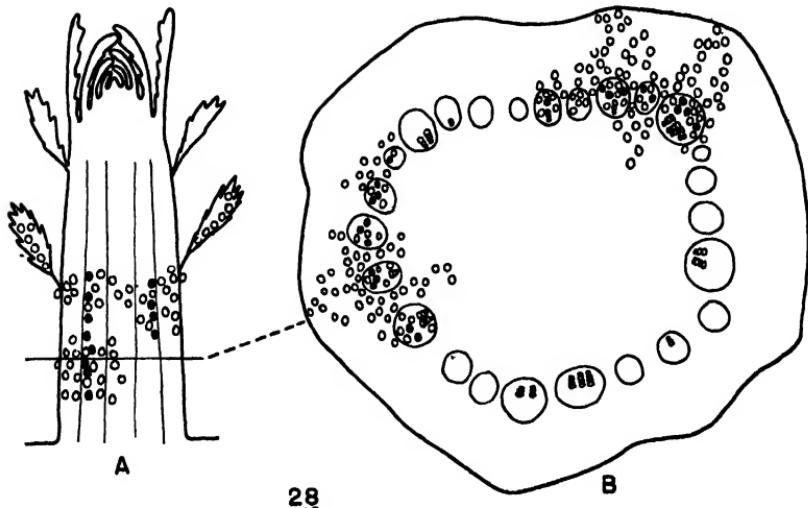
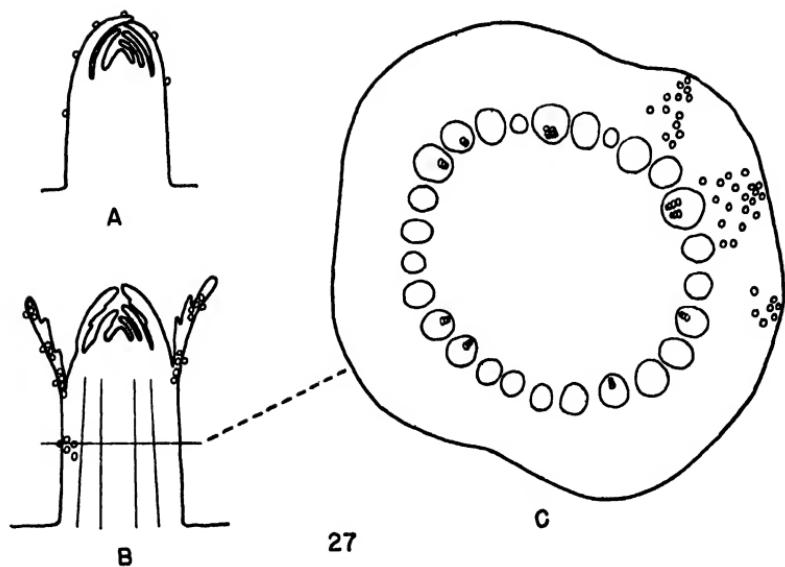


FIG. 27, diagram of 5 day old infection: *A*, shoot at time of inoculation; *B*, diagram of longitudinal section of shoot 5 days later; *C*, cross section of inoculated shoot drawn with camera lucida,  $\times 720$ . (Circles  $\circ\circ\circ$  represent distribution of intracellular mycelium.) ; 28, diagram of 12 day old infection: *A*, inoculated shoot; *B*, cross section  $\times 720$ ;  $\circ\circ\circ$  = intracellular mycelium,  $\bullet\bullet\bullet$  = intercellular mycelium.

## DEVELOPMENT OF THE INTERCELLULAR MYCELIUM

Longitudinal sections through infected shoots ten to twelve days after inoculation show that in the primary phloem a few long strands of intercellular hyphae are present. These strands are to be found only in the bundles in which the intracellular mycelium

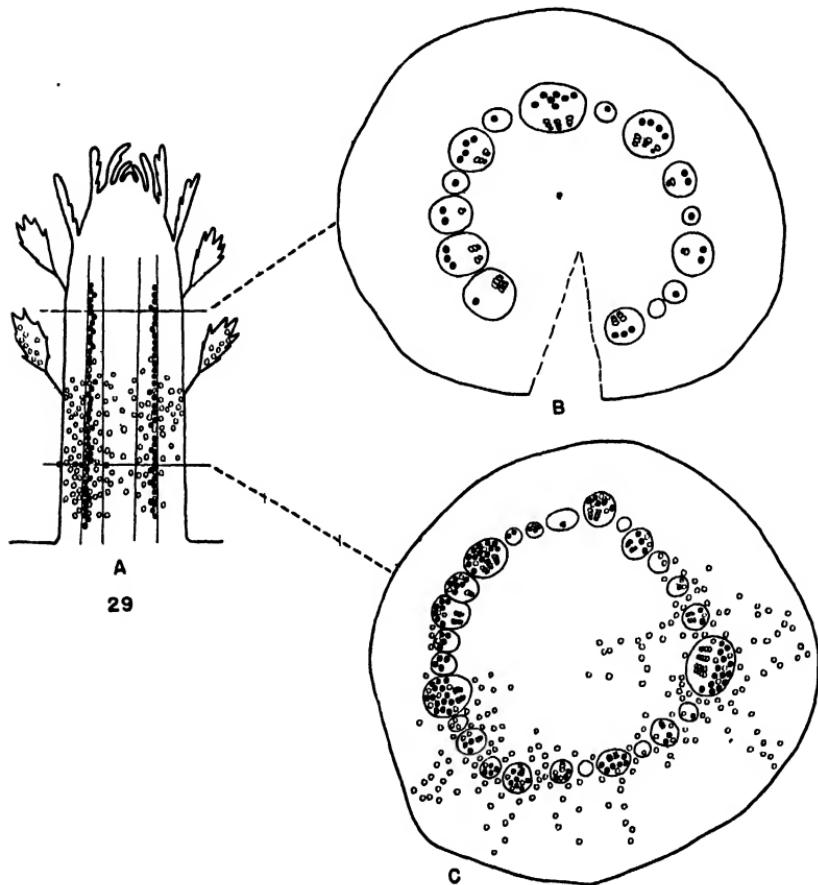


FIG. 29, diagram of 18 day infection: *B*, *C*, are cross sections at two different levels of shoot *A*  $\times 720$ ;  $\circ\circ$  = intracellular mycelium,  $\bullet\bullet$  = intercellular mycelium.

is well established and are represented by the solid black circles (FIG. 28). By the 18th day both kinds of mycelium show a decided increase (FIG. 29). One shoot was analyzed at this stage and the distribution of the mycelium is shown in longitudinal sec-

tion at *A*, and in cross section at *B* and *C*. The intracellular mycelium has invaded practically the entire area at the level from which the section *C* was taken. Intercellular hyphae are present in the phloem of most of the bundles. In the larger bundles the number of strands may be rather high (FIG. 29C). Figure *B* is a cross section from near the tip of the shoot and shows that only the intercellular mycelium is present. This indicates clearly that the intercellular mycelium spreads rapidly upward while the intracellular mycelium is more or less confined to the region of original infection.

In cross section the intercellular hyphae bear a striking resemblance to the so-called companion cells of the host (FIG. 30). They are usually slightly irregular in outline and are generally located in the interstices of the cells. Occasionally, however, they run between the lateral walls (FIG. 30). This drawing, which is from an 18 day old infection, gives the impression that the intercellular hyphae run at a sharp right angle to the plane of the section. That this is not strictly true is indicated in figure 31. Here a few hyphae have been drawn at one particular focus (represented by the dark line) and at a lower level the same hyphae are again shown, this time by a dotted line. It is at once apparent that these hyphae pursue a somewhat irregular course through the phloem. In figure 32 one hypha has been drawn at four successive foci. At *A*, there are two distinct branches. Slightly below *A*, at *B*, they have united into one. At *C* the hypha is running at right angles to the plane of the section and spreading apart the walls of the two adjacent cells. Focusing just below this point we see that at *D* the hypha has again turned and is travelling downward. These figures represent the typical course of the intercellular hyphae in the phloem. In longitudinal section the hyphae are composed of rather short, uninucleate cells (FIG. 33). Haustoria are present in abundance in the well-established hyphal system (FIG. 33, 34). They are long, slender, multicellular and very hypha-like. The tip usually is slightly coiled, the amount of coiling apparently being dependent upon the size and shape of the host cell. In a large cell, as in the pith, the haustoria are more characteristic with a well defined coil at the tip (FIG. 35). This figure recalls very strongly the coil of the intracellular mycelium (FIG. 21).

During the progress of infection the young cane has been elongating rapidly. Dodge (5) has shown that the growing shoot leaves the fungus behind since the growing point is protected by the young leaves and is free from mycelium. Dodge believed that the fungus grew more rapidly downward than upward. A study

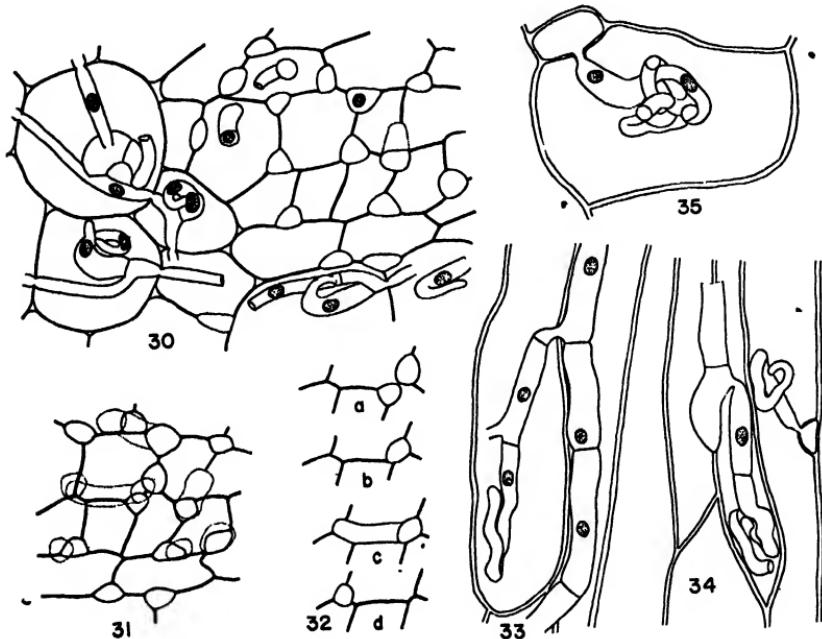


FIG. 30, inter- and intracellular mycelium in cross section, after 18 days; 31, intercellular strands at different levels (upper level shown by heavy line, lower level by dotted line); 32 *a-d*, four views of the same strand at four different foci; 33-34, typical intercellular mycelium in longitudinal section, showing haustoria; 35, haustorium in pith; compare with figure 21. (All figures reduced to 600 X.)

of many shoots seems to indicate that while the mycelium does grow downward it also grows upward a considerable distance, as indicated in figure 29. At the end of 100 days one shoot had reached a height of 12 inches. The extreme tip had become injured and died. Sections revealed that the intercellular mycelium extended the entire length of the living shoot, while intracellular mycelium was found to be present only at the base of the cane. At the point where the cane joined the root both kinds of mycelium were present in abundance. The intercellular mycelium was not

confined to the pith and numerous strands were observed in the cortex. This indicates that in this case, at least, infection was successful since the mycelium was already present in the root system.

#### TRANSITION FROM INTRA- TO INTERCELLULAR MYCELIUM

Intercellular mycelium appears only after the intracellular is well established. The question then arises when, where and how does this change take place. From the foregoing data it may be concluded that the intracellular mycelium gives rise to intercellular hyphae in the phloem of the young bundles, from the 12th day of infection onward. The problem of the method by which this transition occurred was solved by a detailed study of freehand longitudinal sections of an infected shoot. It is possible to find in certain parts of the sections places where both kinds of mycelium are present. The method followed was to trace as far as possible individual intracellular hyphae. By careful search several places were found where the actual transition was taking place.

Six typical cases are shown in figures 36 to 41. In the first of these figures the primary runner instead of passing through the wall and into the next cell has entered the middle lamella, and turned at right angles to give the characteristic appearance of the intercellular hyphae. The secondary runner has continued the growth of the intracellular mycelium in the next cell. Figure 37 illustrates another method. Here the secondary runner has become an intercellular hypha. At *a*, the same hypha is shown at a lower focus. The characteristic wide opening which the runner makes as it enters the wall is shown in figure 38. The wall seems to extend along the runner for a short distance giving it a sheathed appearance. This is also indicated in figures 37, 40, 41. There is never any suggestion of a sheath surrounding the rest of the mycelium in the cell. Evans (9) in *Urocystis Cepulae* found a similar sheath on the penetration hypha near the point of entrance. In figures 39, 40, 41 the primary runner has become intercellular. The different aspects of this change, as one focuses downward, are shown in figure 40. At *a*, *b* and *c* the hypha has been drawn at three different foci; at *a*, making a wide opening in the wall; at *b*, the typical intercellular appearance; at *c*, moving obliquely to the

plane of the section. The young intercellular hypha grows in the middle lamella forcing the two walls apart. In figure 41 the intercellular hypha which has arisen from a primary runner, has grown along in the wall for some distance. In cases such as these the wall on each side of the hypha is clearly seen, and it is exactly one half the thickness of the common wall of the two host cells. It is extremely difficult to find cases of transition in the phloem. The cells are not only very narrow but they usually contain haustoria as well as intracellular mycelium. The cells of the pith and cortex being much larger afford a much better opportunity for studying these details. In these tissues they have been observed to arise from both the primary and secondary runners, but mainly from the former. The first formed intercellular hyphae in the phloem undoubtedly arise just as do those of the cortex and pith.

#### DESTINY OF THE INTRACELLULAR MYCELIUM

During the growing season the young infected plants were kept in pots in the greenhouse. In the fall they were transferred to a cold frame and allowed to rest until the first of February, at which time they were once more placed in the greenhouse. One plant was removed from the pot, brought into the laboratory and carefully studied. This plant was evidently infected since four small buds were present at the base of the cane. The infected cane was about 18 inches in height. Freehand sections revealed intercellular mycelium in phloem and cambium. The tip of the cane had been injured and as a result had died back to a point half an inch above the limit of the intercellular mycelium. All four buds showed vigorous hyphae and haustoria in the growing points. Had these buds been allowed to grow all of the leaves would have been rusted.

At the end of the growing season the intracellular mycelium had been found to be largely confined to the basal part of the cane. It was of interest, therefore, to see what changes may have taken place in this mycelium as a result of overwintering. Sections through the old cane about one inch from the root showed that the intracellular mycelium was still present but did not appear to be in an active state of growth. The hyphae were clearly distinguishable though practically devoid of contents.

Four to five weeks later rusted leaves began to appear on the

inoculated plants in the greenhouse, indicating that the infections had been successful. In figure 42, A, is shown a photograph of one of these infections. At the time of inoculation (May 31st) the shoot had grown about an inch from the root cutting. Free-

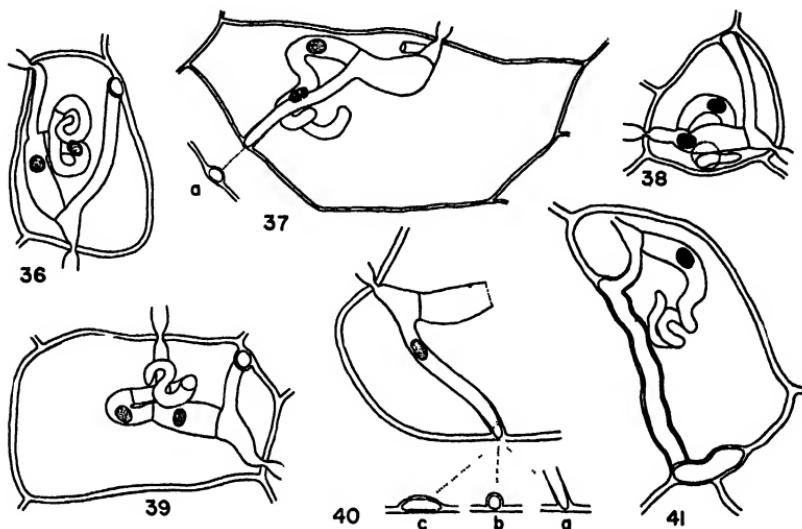


FIG. 36-41, transition from intra- to intercellular mycelium; 36, primary runner becoming intercellular; 37, secondary runner becoming intercellular; at a lower focus, *a*, it is clearly intercellular; 38, wide opening in cell wall and sheath near point of entrance; 39, primary runner becoming intercellular, the secondary runner continuing the intracellular growth; 40, transition of the primary runner at three foci: *a*, entering the cell wall; *b*, same hypha in the wall; *c*, the hypha is running obliquely in cell wall; 41, secondary runner becoming intercellular and running horizontally in the cell wall. (All figures reduced to 600 X.)

hand sections from this and other plants showed very little intracellular mycelium. A few strands were observed in the cortex and these appeared to be in the initial stages of degeneration. The plant illustrated in figure 42, B, shows a young infection on *Rubus occidentalis* with the infected shoots in a dormant condition. Each year the number of new infected shoots will be augmented so that in a few years the plant has assumed a typical witches' broom appearance (FIG. 42, C, D).

Having followed the course of infection from basidiospore penetration to the appearance, a year later, of aecia on the newly opened



FIG. 42

leaves, it becomes possible to study the role and destiny of the intracellular mycelium in the light of the complete story of infection. This mycelium is prominent in the earliest stages and by its penetration of all tissues makes possible the development of the first intercellular strands in the phloem. By its very nature the intracellular mycelium is apparently inadequate to meet the conditions arising in a young growing shoot. Intercellular hyphae, growing longitudinally, are able to keep pace with the rapidly growing cane. At first the intracellular hyphae probably act in an haustorial relation to the intercellular hyphae, but as soon as the latter develops haustoria of its own, it becomes independent. Throughout the growing season, however, new intercellular runners are continually being formed, so that by the end of the growing season a considerable amount of mycelium has been produced. When growth begins again the following spring the intercellular mycelium continues its independent development. There is therefore no further necessity for continued intracellular growth. The evidence seems to indicate that during the following year the mycelium gradually degenerates. At the end of the second year the originally infected cane dies out and with it all traces of the primary infection disappear.

#### DISCUSSION

Intracellular mycelia are not uncommon in other groups of fungi. The mycelium of certain of the Polyporaceae is regularly intracellular, while in the smuts it is often both inter- and intracellular. In the ascomycetes and fungi imperfecti many cases have been reported, while in the phycomycetes intracellular mycelium is well known, as for instance in *Rhizopus* on strawberries. Miss Rice (15) has reviewed the literature on intracellular parasites up to 1927 and an extensive review is not necessary here. The significant thing about the majority of the intracellular forms, as compared with the present case, is that the mycelium runs from cell to cell with little or no branching, and without notable differentiation. The opening in the cell wall through which the fungus passes is often a broad one, sometimes being even larger than the diameter of the hypha (11). The intracellular mycelium here reported differs radically in that it is highly differentiated in each cell. This

differentiation is so constant and characteristic that it can be readily identified even when haustoria of the intercellular mycelium are present in the same cell.

Intracellular mycelium is not necessarily associated with saprophytism. It has been reported in the Auriculariales in *Eocronartium* by Fitzpatrick (10), a species which shows a high degree of parasitism. In the rusts, however, so far as I have found, intracellular mycelium has not been thus far reported. The rusts are characterized by intercellular mycelia with haustoria in the cells of the host. Apparently an especially delicate balance between the parasite and the host is established under these conditions. The presence of a well developed mycelium entirely within the cells might result in an even more intimate relationship, such as is found in mycorrhiza and in certain chytrids. It would be very desirable to make a cytological study of the host-parasite relations in connection with the intracellular mycelium in *Gymnoconia*.

In those rusts in which the development of the haploid mycelium has been studied there is a brief intracellular phase which is usually confined to a single cell. The penetration hypha of the basidiospore is generally intracellular, and often considerably branched (2, 3). In *Endophyllum Sempervivi* the situation has recently been shown by Miss Ashworth (4) to be similar. The writer, however, has observed that the penetration hypha sometimes forms an extensive mycelium in the large epidermal cells of *Sempervivum*. Hyphae have been observed to follow the complete circumference of the host cell, as if seeking a point of egress. These hyphae are multicellular and this case may be considered as that of a very localized intracellular mycelium. Miss Allen in one of her cytological studies on heterothallism (3) has reported that occasionally receptive hyphae are intracellular. The aeciospores of the long cycled strain of *Gymnoconia interstitialis* penetrate the leaf with a short intracellular penetration hypha which arises from an appressorium (13). The teliospores of the genera *Milesia*, *Calyptospora*, *Hyalopsora* and *Thecopspora* of the Pucciniastreæ are intracellular in the epidermis of their hosts (14).

In many rusts the haustoria are long, filamentous, multicellular and branching. Sometimes the lumen of the cell is filled (5). Haustoria of this type have also been observed by the writer in

*Endophyllum Sempervivi* and in *Puccinia curtipes*. In these cases the haustorium is simply a localized intracellular mycelium. If the haustorium should grow into adjacent cells the situation would be somewhat analogous to that of the intracellular mycelium here reported for the orange-rust. Miss Allen (1), in noting the somewhat mycelium-like nature of the primary infection hypha, states: "It is probable, however, that all of these intracellular growths, whether haustorial in form or intermediate between that and ordinary hyphae, are haustorial in function." The haustorial nature of the intracellular mycelium is at once suggested when comparisons are made with typical haustoria (FIG. 21, 35). The coiled tip of the intracellular mycelium is similar in appearance to the regular haustorium. The two basal cells are formed in connection with the development of the runners.

The intercellular condition supervenes as soon as the phloem has been reached. The cause of this change involves some very interesting problems in parasitism. Both mycelia continue their growth, often side by side, and intercellular strands are continually being formed. Even after haustoria have been developed the intracellular mycelium continues to grow although it appears to slow down as the season progresses. The important fact in this type of infection is that the intercellular mycelium apparently must reach the roots before the end of the growing season. The intracellular mycelium is perhaps the most rapid means of reaching the phloem from the exterior. The ability of the runners to become intercellular and the general downward growth of the mycelium throughout the season, are factors of primary importance in the establishment of the systemic infection.

#### SUMMARY

Basidiospores of the two short cycled strains of the orange-rust, *Gymnoconia interstitialis*, when sown on young shoots of *Rubus*, penetrate the epidermis and form a typical penetration hypha.

From this hypha intracellular branches arise which enter adjacent cells passing through the side walls of the host cell. As the hypha enters the next cell, it cuts off a tip cell. The sub-terminal cell gives rise to a branch called the primary runner. The

terminal cell divides again and this sub-terminal cell also gives rise to a runner called the secondary runner. This cell is much enlarged with a characteristic rounded base. The terminal cell continues to grow, becoming more or less compactly coiled and multicellular. The runners enter neighbouring cells and each repeats this procedure. The result is a highly characteristic intracellular mycelium.

The mycelium grows in this way through the cortex into the vascular bundles and into the pith. From the 10th day onward and continuing throughout the season strands of intercellular mycelium begin to appear in the phloem. These arise from one of the runners, usually the primary runner, which grows into the middle lamella and the intercellular spaces. The intercellular mycelium grows rapidly in the phloem and becomes established as a perennial mycelium in the cane and in the roots.

The function of the intracellular mycelium seems to be that of establishing the fungus in the host, and is probably haustorial in nature. Little, if any, growth of the intracellular mycelium takes place the following spring and the evidence indicates that degeneration takes place later.

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#### EXPLANATION OF FIG. 42

FIG. 42, *A*, one of the infected plants of blackberry a year after inoculation (note the new shoots at the base of the cane); *B*, dormant infected plant of *Rubus occidentalis* from nature; this plant having not been long infected, as indicated by the small number of new shoots; *C-D*, photograph of the crowns of old established infections on blackberry showing the many new shoots in a dormant condition, when leaves mature on these shoots they will all be rusted. (The above-ground parts form a characteristic witches' broom.)

# SPUMULA, A NEW GENUS OF RUSTS

E. B. MAINS<sup>1</sup>

(WITH 1 FIGURE)

While consulting phanerogamic specimens in the Herbarium of the University of Michigan, a rust was noted on a specimen of *Calliandra bijuga* Rose which had been collected by Ynes Mexia in Jalisco, Mexico, February 4, 1927. An examination showed that this presented some very unusual features. After a comparison with related species of rust, it has been concluded that it is an undescribed species belonging to a new genus for which the name *Spumula* is proposed.

## Spumula gen. nov.

*Aecia subepidermalia, poculiformia, peridio instructa; telia subepidermalia, pulverulentia; teliosporae hemisphaericæ, compositæ ex 4 (raro 3) cellulis a latere conjunctis; cystidia semper fere 4, globosa pendula; pedicellus longus, ex una hypha compositus.*

**Spumula quadrifida** sp. nov. *aeciis minutis 0.2–0.3 mm., hypophyllis, sparsis vel aggregatis, poculiformibus vel cylindricis; cellulis peridii rhomboideis, 14–20 × 20–26 μ, dense minuteque verrucularis, pariete exteriore 5–6 μ crassa, interiore 2–2.5 μ; aeciosporis late ellipsoideis, 12–16 × 16–22 μ, minutissime verruculosis, membrana hyalina 1 μ crassa; teliis hypophyllis, sparsis vel circum aecia aggregatis, pulverulentis, fulvis; teliosporis levibus, 42–54 μ latis, 26–30 μ crassis, ex 4 cellulis (raro 3) compositis, fulvis; cystidiis 4, hyalinis, 10–14 × 16–26 μ; pedicellis fragilibus, hyalinis, quibusque ex una hypha compositis.* (FIG. 1 A, B.)

In foliis Caliandrae bijugae. Legit Ynes Mexia (1638 A), inter San Sebastian et Real Alto, Jalisco, Mexico, Feb. 4, 1927. In Herbario Universitatis Michiganensis conservatum.

*Aecia hypophylloous, scattered or in groups of two or three, cupulate to cylindric, small, 0.2–0.3 mm. in diameter; peridial cells rhombic in side view, 14–20 × 20–26 μ, the outer wall 5–6 μ thick, the inner 2–2.5 μ, finely and closely verrucose; aeciospores broadly ellipsoid 12–16 × 16–22 μ, the wall colorless, 1 μ thick, very finely verrucose. Telia hypophylloous, scattered, often closely associated*

<sup>1</sup> Papers of the Department of Botany and the Herbarium of the University of Michigan, no. 540.

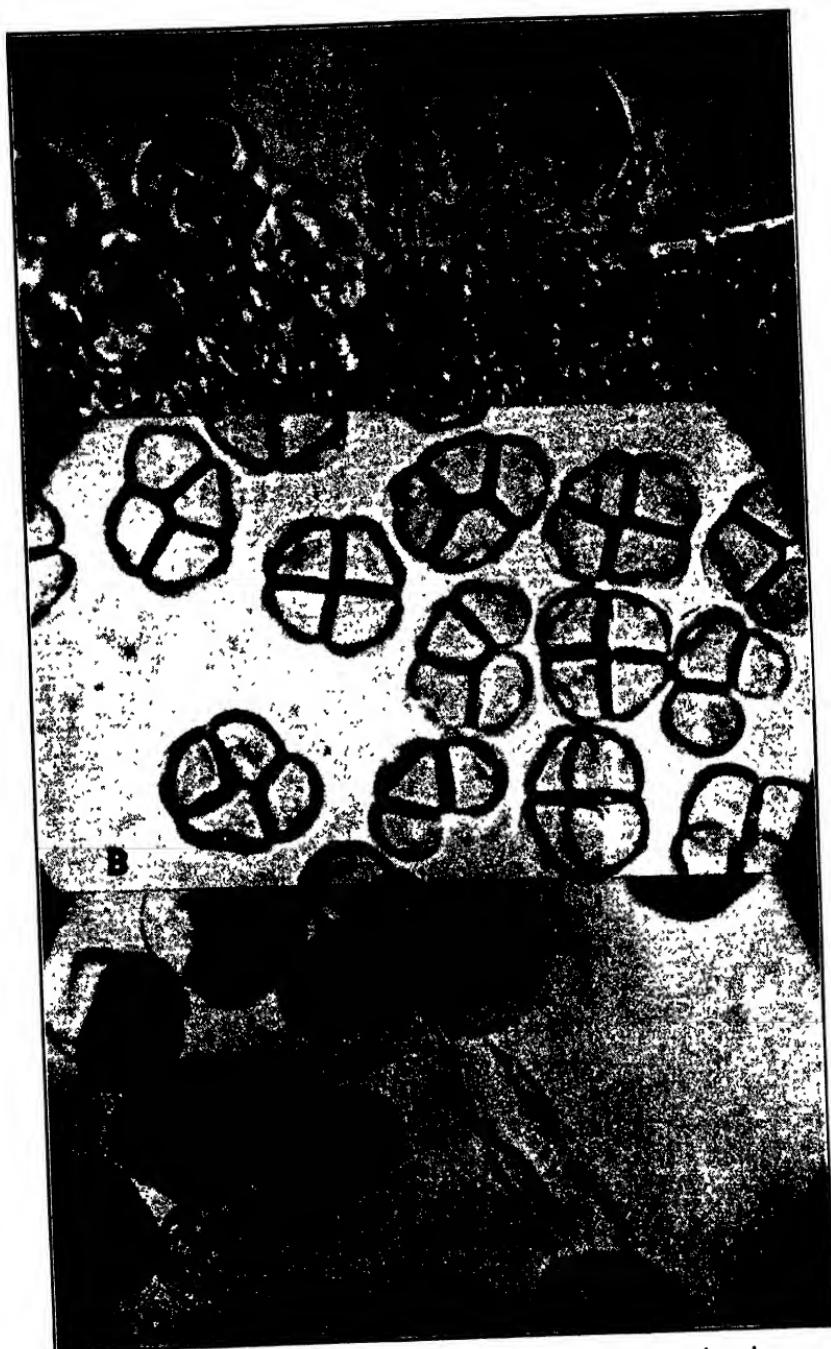


FIG. 1. A, B, *Spumula quadrifida*; C, *Cystomyces costaricensis*.

with the aecia; teliospores sometimes developing in old aecia, pulverulent, cinnamon-brown; teliospores smooth, 42–54  $\mu$  in diameter, 26–30  $\mu$  thick, usually consisting of four cells, the walls golden brown, 1.5–2  $\mu$  thick, the germpore covered with hyaline thickening, 4–6  $\mu$ , cysts hyaline, usually one to each cell, 10–14  $\times$  16–26  $\mu$ ; pedicel fragile, colorless, simple.

On *Calliandra bijuga* Rose, trail from San Sebastian to Real Alto, Jalisco, Mexico, Ynes Mexia (1638, type), Feb. 4, 1927.

The pulverulent telia, due to the fragile pedicels of the teliospores, superficially have the appearance of uredinia. Uredinia, however, are lacking. The small aecia are scattered or in groups of two or three. They are small and inconspicuous. They are not accompanied by pycnia indicating that they may be repeating aecia. Several were found to contain teliospores in addition to aeciospores. The teliospores are mostly four-celled, the cells laterally united into a head. A hyaline cyst is more or less pendant beneath each cell. A number of teliospores were found with basidia indicating that germination probably occurs without a long rest period.

This rust might be considered a simple *Ravenelia* in which no fusion has occurred. In this respect *Ravenelia simplex* Diet. approaches it. Although the latter species has some simple spores, most are compound as in the majority of species of *Ravenelia*. In *Spumula quadrifida* the pedicel of the teliospore is a single hypha and there is no evidence of a fusion of teliospores.

The simple pedicel immediately suggests the genus *Cystomyces*. At present only one species is known, *Cystomyces costaricensis* Syd. This is a short-cycled species. Both Sydow and Dietel<sup>1</sup> have emphasized the occurrence of a simple pedicel in separating this genus from *Ravenelia*. Through the kindness of Dr. David H. Linder it has been possible to examine material of *C. costaricensis* from the Farlow Herbarium. The structure of the teliospore of *Cystomyces* differs markedly from that of *Spumula*. In *Cystomyces costaricensis* the spore is formed of three dark colored cells laterally united. Below there are three hyaline cysts also

<sup>1</sup> Sydow, H. Fungi in itinere costaricensi collecti. Ann. Myc. 24: 290–292. 1926. Dietel, P. Uredinales. Engler—Prantl Natürlichen Pflanzenfamilien 2 Auf. 6: 70–71. 1928.

closely united, the whole forming a somewhat irregular globoid head (FIG. 1C). The simple pedicel is attached to the cysts and not to the dark colored cells as in *Spumula*. The differences are illustrated in figure 1. *Spumula* is apparently more closely related to *Ravenelia* than to *Cystomycetes*.

#### EXPLANATION OF FIGURE

*A*, *Spumula quadrifida*, side view of teliospore (Note simple pedicel and pendant cysts); *B*, *Spumula quadrifida* showing quadrifid horizontal arrangement of cells of the teliospores; *C*, *Cystomycetes costaricensis* (Sydow, Fungi exotici exsiccati 595), contrast with *A* and *B* (Note attachment of pedicel to the coherent cysts); all  $\times 440$ .

# NEW OR NOTEWORTHY BASIDIOMYCETES FROM THE CENTRAL ROCKY MOUNTAIN REGION

FRED J. SEAVER AND P. F. SHOPE

(WITH 4 FIGURES)

This paper deals with some new or noteworthy species of Basidiomycetes collected in Colorado and Wyoming during the summer of 1929 by the senior author from the New York Botanical Garden, and the junior author from the University of Colorado and a resident of Colorado. A brief synopsis of the activities of the authors during the summer of 1929 has previously been published (7). Also, a report covering the rusts which were collected has already been made (9).

Based entirely or in part upon the Basidiomycetes collected in 1929, one new genus and two new species have already been reported, as follows: *Calbovista subsculpta* Morse (*Mycologia* 27: 97, 1935) and *Phlebia mellea* Overholts (*Mycologia* 22: 241, 1930). This present paper presents one additional new species.

Complete and annotated check-lists of all the Basidiomycetes, except the rusts, collected by the authors in Colorado, Wyoming, and South Dakota, will appear in the "University of Colorado Studies," Volume 23, number 2. 1935. This paper deals with one new species, and other species of interest in respect to their geographical and altitudinal distribution, host relations, and morphological characteristics.

## DACRYOMYCETACEAE

**DACRYOMYCES ABIETINUS** (Pers.) Schröt. On dead decorticated *Picea Engelmanni*. Middle Boulder Canyon, Colo., No. 25 and 53; University of Colorado Summer Camp, No. 146. Also, collected in Colorado by the junior author in 1927 and 1928 on the same host. This is the first report of this species from Colorado and from the Rocky Mountain States. Dur-

ing the summer and autumn of wet years, this species is of frequent occurrence.

**HETEROTEXTUS ALPINUS** (Tracy & Earle) Martin, Mycologia **24**: 217. 1932.

*Guepinia alpina* Tracy & Earle, in Greene, Pl. Bakerianae **1**: 23. 1901.

*Guepinia monticola* Tracy & Earle, *ibid.*

*Ditiola Shopei* Coker, Jour. Elisha Mitch. Soc. **46**: 117. 1930.

On dead *Picea Engelmanni*. Middle Boulder Canyon, Colo., No. 2.

This is a common species in the Central Rocky Mountain region in wet springs and summers. During late summer and autumn, when conditions of moisture are inadequate, this species is frequently rare.

The junior author has noted that the distribution of this species in Central Colorado extends from approximately 7,000 feet to 11,300 feet in elevation, and it is most abundant at elevations of from 9,000 feet to 11,000 feet.

Young fructifications are almost globose; later, they become cup-shaped, with the hymenium either plane, concave, or convex depending upon the abundance of moisture. They have been found to be as large as 14 mm. in diameter. The hymenium is most always superior.

#### TREMELLACEAE

**TREMELLODON GELATINOSUM** (Scop.) Pers. On dead *Picea Engelmanni*. North Park, Colo., elevation 10,000 feet, No. 535; and Grand Mesa, Colo., elevation 9,000 feet, No. 558.

This is the first report of this species from Colorado and the Rocky Mountain States. A large and excellent collection of this interesting fungus was made at Grand Mesa in September following a rainy period of over one week in duration (FIG. 4).

Other interesting tremellaceous fungi collected in Colorado and not previously reported from either Colorado or the Rocky Mountain States are: *Tremella lutescens* Pers. On dead *Populus tremuloides*, Tolland No. 469; and *Tremella pinicola* Beritz. On dead *Picea Engelmanni*, Pikes Peak, No. 458.

## THELEPHORACEAE

ALEURODISCUS AMORPHUS (Pers.) Raben. On twigs of dead *Picea Engelmanni*. Collected by Dr. E. Bartholomew and the junior author at Tolland, Colo., No. 471 (FIG. 1a).



FIG. 1. a, *Aleurodiscus amorphus*; b, *Clavaria apiculata*; c, *Merulius fugax*.

A previous collection of this fungus made by the junior author in Colorado in 1928, was sent to Dr. L. O. Overholts, and reported by him (Mycologia 25: 426. 1933). This species is also known to occur in Idaho, and its discovery in Colorado points to its distribution throughout the Rocky Mountain system.

**CORTICIUM LACTEUM** Fries. On dead decorticated *Picea Engelmanni*. University of Wyoming Summer Camp, No. 195. First report from Wyoming. Previously known to occur in the Rocky Mountain States in Idaho and Colorado.

**CYPHELLO FASCICULATA** (Schw.) Berk. & Curt. On dead *Salix* sp. University of Colorado Summer Camp, No. 95. New host. Previously known to occur chiefly on *Alnus*, and rarely on *Prunus* and *Pyrus*. Identified by Dr. L. O. Overholts.

This fungus has a wide distribution in Eastern United States with Wisconsin and Alabama representing the western limits of



FIG. 2. *a*, *Peniophora carnosa*; *b*, *Stereum rugosporum*.

its distribution. Several collections of this species have recently been reported from Oregon (3, 11).

The discovery of this species in Colorado represents the first collection from the Rocky Mountain States and west of Wisconsin and Alabama.

**PENIOPHORA CARNOSA** Burt. On dead *Populus tremuloides*. University of Wyoming Summer Camp, No. 200. The junior author in 1928 collected this fungus in Colorado on the above host (Shope Herb. No. 450) (FIG. 2a).

This species is usually found on the bark and wood of coniferous logs, rarely on frondose species. Since this fungus has previously been reported from Montana, Idaho, and New Mexico by Dr. Burt (2), its discovery in Wyoming and Colorado in abundance further proves Dr. Burt's statement: "Common in the Rocky Mountain forests" (l. c.).

**STEREUM RUGISPORUM** (Ellis & Ev.) Burt. "On dead *Picea Engelmanni*. Near Brooklyn Lake, Wyo., elevation 10,500 feet. No. 239; and same host, Middle Boulder Canyon, Colo., elevation 10,000 feet, No. 58 (FIG. 2b).

As reported by Dr. Burt (1), this species is of common occurrence in the Rocky Mountain States on various species of conifers. In moist years it is abundant. It is found most frequently at elevations of from 10,000 feet to 11,000 feet. The junior author is of the opinion that this species is occasionally perennial.

#### POLYPÓRACEAE

Our collections of pore-fungi in Colorado yielded nothing of outstanding interest that has not already been reported by the junior author in a previous publication (8). The pore-fungi of Wyoming, however, have received very little consideration in our literature. Therefore, the few species collected in that state throw some light upon the distribution of these species in the Rocky Mountain States. All species collected in Wyoming occur also in Colorado. *Fomes Pini* (Thore) Lloyd, *Polyporus alboluteus* Ellis & Ev., and *P. leucospongia* Cooke & Hark. occur in Colorado, Wyoming, and according to Dr. Weir (10) in Montana. *Polyporus circinatus* Fries and *P. ursinus* Lloyd, however, seem to reach their northern limit of distribution in Wyoming, since they have not been reported from Montana (l. c.).

MERULIUS FUGAX Fries. On dead *Pinus Murrayana*. Grand Mesa, Colo., No. 578. First report from Colorado (FIG. 1c).

This fungus is known to occur in Idaho, and its occurrence in Colorado points to its distribution throughout the Rocky Mountain region. The junior author knows of three collections of this species from Colorado all of which were made from the same type of host.

#### CLAVARIACEAE

CLAVARIA APICULATA Fries. On rotted coniferous wood. Middle Boulder Canyon, Colo., No. 55; and Grand Mesa, Colo., No. 560 (FIG. 1b).

Dr. Coker (4) reports this fungus as occurring in the Eastern States and Washington. This Colorado collection is apparently the first report of the occurrence of this species in the Central United States and the Rocky Mountain region.

#### AGARICACEAE

AMANITA MUSCARIA (L.) Fries. On the ground under conifers. University of Wyoming Summer Camp, No. 196; and Tolland, Colo., No. 477.

In a recent article entitled, "Mycofloristic impressions of a European mycologist in America," by Mr. J. E. Lange (6), he notes that: "While in Europe *Amanita muscaria* is almost always bright scarlet—, all over the Eastern States a similar species but with a clear orange-yellow hue occurs. The existence of this orange-colored American form is the more remarkable because the scarlet European type also occurs in certain parts (*e.g.* the eastern Canadian provinces and Oregon, where it is said to attain gigantic dimensions)." Dr. Kauffman was the first to point out the occurrence of this scarlet-colored fly-mushroom in Colorado (5), and the authors of this paper found again this agaric with the typical coloration of the European plant. This scarlet-colored form was found also in Wyoming, and thus indications point to its still wider distribution in the Rocky Mountain region. Although Dr. Kauffman's collections in Colorado, as well as our collections from Colorado and Wyoming, were made at elevations of from 9,000 feet to 10,000 feet, this does not indicate that the

scarlet-colored form occurs in the United States only at relatively high elevations. Dr. Zeller has collected this scarlet form in Oregon in pine barrens along the coast (12).

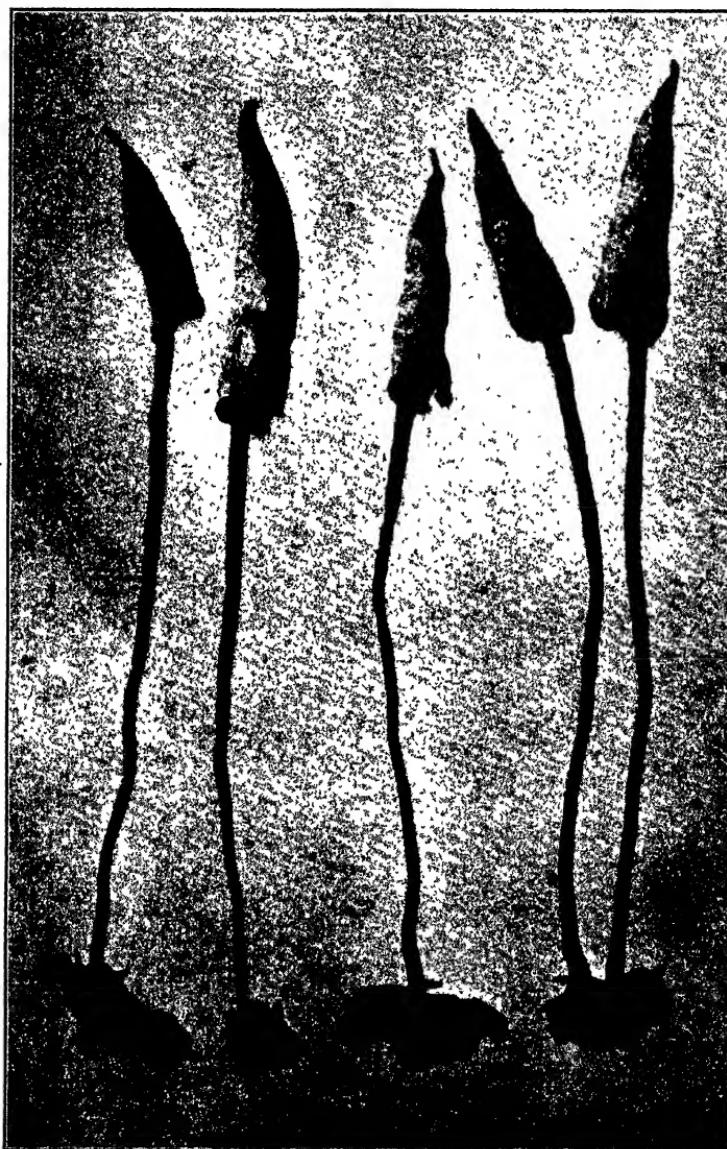


FIG. 3. *Bolbitius cucullatus*.

**Bolbitius cucullatus** Shope & Seaver, sp. nov. (FIG. 3).

Pileus 6–10 mm. broad, 25–40 mm. high, conical, not expanding, cinereous to pale yellow when fresh, drying Cinnamon-Buff (Ridg.) to Clay Color (Ridg.), subviscid, even, glabrous; apex cuspidate, concolorous; margin inturned or straight, acute, lacerate with age, concolorous; context less than 1 mm. thick, whitish; lamellae adnexed, close, 0.5–2 mm. broad, whitish, with age becoming brownish-ochraceous, drying Vandyke Brown (Ridg.), dissolving slightly with age; stipe 10–12 cm. long, 1–2 mm. thick, equal, flaccid, glabrous to slightly floccose, stuffed or hollow, concolorous with the pileus, drying striate, bulbous at the base; spores ellipsoid to ovoid, sometimes apiculate, smooth, ochraceous under the microscope,  $12-16 \times 8-10 \mu$ .



FIG. 4. *Tremellodon gelatinosum*.

Pileo tenerrimo, elongato-conico, levi, subviscido, cinereo vel flavo, apice cuspidato aut cucullato, 6–10 mm. lato, 25–40 mm. longo; stipite gracile, fistuloso, basi leviter incrassato, 1–2 mm. lato, 10–12 cm. longo; sporis ellipsoideis vel ovoideis, saepe apiculatis, ochraceis,  $8-10 \times 12-16 \mu$ .

Type collection No. 249. Gregarious to scattered. Among grass near the University of Wyoming Summer Camp, elevation 9,600 feet, August 2, 1929. Known only from the type collection.

This new species is well marked and distinct. Its closest affiliation is *Bolbitius tener* Berk., to which it is related by its similar spore and hymenial characteristics. It differs from *B. tener* in that the upper part of the pileus is drawn out into a cusp; the pileus does not finally expand; the stipe may at times be stuffed, and the new species is more than twice the size of the former species. About fifty specimens were collected and several hundred others observed all of which had the characteristics that are described above.

#### LYCOPERDACEAE

**CALBOVISTA SUBSCULPTA** Morse, Mycologia 27: 97. 1935. On the ground under conifers. Middle Boulder Canyon, Colo., elevation 10,000 feet, July 23-28, No. 36; and University of Wyoming Summer Camp, elevation 9,600 feet, August 1-3; No. 192. This latter collection from Wyoming was not mentioned in Miss Morse's paper, and it adds another state to the range which to date includes California, Washington, Idaho, Colorado, and Wyoming. This fungus is evidently found only at high elevations throughout the Rocky Mountain region and the Western Coastal ranges.

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#### EXPLANATION OF FIGURES

Fig. 1. *a*, *Alcurodiscus amorphus* (Pers.) Raben.; *b*, *Clavaria apiculata* Fries; *c*, *Merulius fugax* Fries; all natural size.

Fig. 2. *a*, *Peniophora carnosa* Burt; *b*, *Stereum rugosporum* (Ellis & Ev.) Burt; both  $\frac{1}{2}$  natural size.

Fig. 3. *Bolbitius cucullatus* Shope & Seaver, natural size.

Fig. 4. *Tremellodon gelatinosum* (Scop.) Pers.,  $\frac{1}{2}$  nature size.

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